MANGO (*Mangifera indica* L.) KERNEL FAT: FATTY ACID PROFILE, OXIDATIVE STABILITY AND DEVELOPMENT OF FOURIER TRANSFORM NEAR INFRARED (FT-NIR) SPECTROSCOPY CALIBRATION MODELS

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

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ABSTRACT

The oxidative stability of crude, cold-pressed mango kernel fat (MKF) was determined over a period of 240 days using the peroxide value (PV), conjugated diene value (CD) and *p*-anisidine value (AV) tests. The changes in fatty acid profile were monitored with gas chromatography and the oxidative status of MKF effectively predicted by FT-NIR spectroscopy. Results obtained from the different methods complemented each other and indicated the stable character of mango kernel fat against oxidative deterioration.

The fatty acid profile constituted palmitic acid (C16:0; 8.43%), stearic acid (C18:0; 34.98%), oleic acid (C18:1 *cis*; 48.05%), linoleic acid (C18:2; 6.60%) and arachidic acid (C20:0; 1.73%). Trace amounts of C16:1 (0.56%), C18:1 *trans* (0.25%), C18:3 (0.43%), C20:1 (0.25%) and C22:0 (0.40%) were also found.

The freshly pressed MKF had a peroxide value of 2.7 meq.kg^{-1}, CD value of 0.07% and an AV of 2.2 mmol.kg^{-1}. After 40 days of storage, the peroxide values of MKF stored with and without exposure to a limited amount of oxygen at 5, 15, 25 and 40°C increased to 5 meq.kg^{-1} and 4 meq.kg^{-1} respectively. Emulsification of MKF had a stabilising effect (maximum PV = 2.8 meq.kg^{-1}), while exposure to UV light had a catalysing effect (maximum PV = 5 meq.kg^{-1}). These maximum values, decreased after 40 days. The CD values of MKF samples stored with and without exposure to oxygen at 5, 15, 25 and 40 °C increased to 0.18% and 0.16%, respectively at day 40. The CD values of samples exposed to light increased to 0.20% and the emulsified samples showed similar values to that of the MKF samples not exposed to oxygen. The conjugated diene values remained stable after day 40. The *p*-anisidine values of the MKF samples both stored with and without exposure to oxygen at 5, 15, 25 and 40°C varied between 0.5 and 5 mmol.kg^{-1}. The weak correlation to the measurement of nonanal, as well as the low levels of 2-alkenals produced by the MKF, resulted in these low and sometimes non-linear values.

The peroxide, conjugated diene and *p*-anisidine values obtained for MKF stored at 25°C over 240 days were low due to the low content of polyunsaturated fatty acids in MKF. This compared favourably with the higher values attained for sunflower, canola and olive oil, which are all rich in polyunsaturated fatty acids.

The minimal changes observed in the fatty acid profile of mango kernel fat indicated the stability of the saturated fatty acids (C16:0 and C18:0) and oleic acid. In

addition, the instability of linoleic and linolenic acids was evident due to oxidative deterioration. A decrease of 7.41% and 12.80% was observed between day 0 and 240 for the C18:2/C16:0 and C18:2/C18:0 ratios respectively.

The prediction of the oxidative status of the MKF samples by near infrared spectroscopy were possible after the development of calibration models from a total data set of 300 samples of which one-third was used for independent validation. Principle component analysis (PCA) indicated classification at 0, 40 and the remaining (80 – 240) days. The best calibration model for PV yielded a SEP (standard error of prediction) of 0.46 meq.kg^{-1}, correlation coefficient (r) of 0.95, bias of 0.02 and a root mean square error of prediction (RMSEP) of 0.46 meq.kg^{-1}. The CD calibration model had a correlation coefficient of 0.89, SEP of 0.01%, bias of 0.001 and RMSEP of 0.01% when developed on a data set with no pre-processing applied. The AV calibration had a SEP of 0.32 mmol.kg^{-1}, bias of 0.03, RMSEP of 0.32 mmol.kg^{-1} and r of 0.93. The C18:2 and C18:3 models were built using partial least squares (PLS) regression and the values obtained for SEP were 0.31% and 0.054%, RMSEP 0.32% and 0.05%, bias 0.05 and 0.01 and correlation coefficcients were 0.82 and 0.54 respectively. The calibrations for C18:1, C18:0 and C16:0 yielded weaker correlations. Good correlations were obtained when calibrating the C18:2/C16:0 and C18:2/C18:0 ratios.

UITTREKSEL

Die oksidatiewe stabiliteit van ru, koud-geperste mango kern vet (MKV) (*Mangifera indica* L.) is oor 'n periode van 240 dae bepaal deur gebruik te maak van die peroksiedwaarde (PV), gekonjugeerde dieen waarde (CD) en *p*-anisidien waarde (AV) toetse. Die veranderinge in die vetsuurprofiel is gemonitor deur gaschromatografie en die oksidatiewe status van MKV is akkuraat voorspel word deur Fourier transformasie naby infrarooi (FT-NIR) spektroskopie. Die resultate van die verskillende toetsmetodes komplementeer mekaar goed en dui die stabiliteit van mango kern vet teen oksidatiewe verval aan.

Die vetsuurprofiel is saamgestel uit palimitiensuur (C16:0; 8.43%), steariensuur (C18:0; 34.98%), oleïensuur (C18:1 *cis*; 48.05%), linoleïensuur (C18:2; 6.60%) en aragiedsuur (20:0; 1.73%). Spoorhoeveelhede C16:1 (0.56%), C18:1 *trans* (0.25%), C18:3 (0.43%), C20:1 (0.25%) en C22:0 (0.40%) is ook geïdentifiseer.

Die vars geperste MKF het 'n peroksiedwaarde van 2.7 meq.kg^{-1}, 'n CD waarde van 0.07% en 'n AV waarde van 2.2 mmol.kg^{-1} getoon. Na afloop van 40 dae opbergingsperiode by 5, 15, 25 en 40°C het die PV van MKV met 'n beperkte blootstelling aan suurstof na 5 meq.kg^{-1} vermeerder, terwyl die waardes van monsters sonder suurstofblootstelling na 4 meq.kg^{-1} vermeerder het. Emulsifisering van MKV het 'n stabiliserende effek (maksimum PV = 2.8 meq.kg^{-1}) terwyl blootstelling aan ultraviolet (UV) lig 'n kataliserende effek (maksimum PV = meq.kg^{-}1) op oksidasie gehad het. Hierdie maksimum waardes het na 40 dae afgeneem. Die CD waardes van MKF monsters opgeberg by 5, 15, 25 en 40°C en met beperkte blootstelling aan suurstof het vermeerder tot 0.18% terwyl die monsters sonder suurstofblootstelling by bogenoemde temperature vermeerder het tot 0.16% na 40 dae. Die gekonjugeerde dieen waardes van die monsters blootgestel aan UV lig het vermeerder tot 0.20%; terwyl die geëmulsifiseerde monsters waardes soortgelyk aan die MKV monsters sonder blootstelling aan suurstof getoon het. Gekonjugeerde dieen waardes het gestabiliseer vanaf dag 40. Die *p*-anisidienwaardes van MKV monsters opgeberg by temperature van 5,15, 25 en 40°C, met en sonder blootstelling aan suurstof, het varieer tussen 0.5 en 5 mmol.kg^{-1}. Die swak korrellasie tussen nonanal produksie en *p*-anisidienwaardes, sowel as die klein hoeveelhede 2-alkenale geproduseer, was verantwoordelik vir hierdie lae en nie linêere waardes.

Die peroksied, gekonjugeerde dieen en *p*-anisidienwaardes wat verkry is nadat MKV by 25°C in 240 opgeberg is, was laag weens die klein persentasie poli-onversadigde vetsure teenwoordig in die vet. Dit vergelyk goed met die hoë waardes wat verkry is vir sonneblom-, canola- en olyfolie wat almal ryk aan poli-onversadigde vetsure is.

Die minimale veranderinge in die vetsuurprofiel van MKF dui op die stabiliserende invloed van versadigde vetsure (C16:0 en C18:0) en oleïensuur. Die onstabiliteit van linoleïen- en lineensuur duidelik uit hierdie vetsure se oksidatiewe verval. 'n Afname van 7.41% en 12.80% is waargeneem tussen dae 0 en 240 vir die C18:2/C16:0 en C18:2/C18:0 verhoudings, onderskeidelik.

Die voorspelling van die oksidatiewe status van die MKF monsters met behulp van FT-NIR spektroskopie was moontlik deur die ontwikkeling van kalibrasie modelle. 'n Totale datastel van 300 monsters, waarvan ongeveer 'n derde vir validasie aangewend is, is gebruik vir die kalibrasiemodelle. Met behulp van PCA (*hoofkomponent analise*) kon drie klassifiseerbare groepe by 0, 40 en 80-240 dae onderskei word. Die beste kalibrasiemodel vir PV het 'n standaardfout van voorspelling (SEP) van 0.46 meq.kg^{-1}, 'n korrellasiekoëffisient (r) van 0.95, 'n oorhelling van 0.02 en 'n standaardfout van voorspelling (RMSEP) van 0.46 meq.kg^{-1} gehad. Die CD kalibrasiemodel (geen voorafverwerking) het 'n r van 0.89, SEP van 0.01% oorhelling van 0.001 en RMSEP van 0.01% gehad. Die AV kalibrasie het 'n SEP van 0.32 mmol.kg^{-1}, oorhelling van 0.03, RMSEP van 0.32 mmol.kg^{-1} en r van 0.93 gehad. Die C18:2 en C18:3 modelle is saamgestel deur PLS (*partial least squares*) regressie. Waardes verkry vir C18:2 en C18:3 was onderskeidelik: SEP 0.32% en 0.05%, RMSEP, 0.32% en 0.05%, oorhelling 0.05 en 0.01 en r 0.82 en 0.54. In die geval van C18:1, C18:0 en C16:0, het die kalibrasies swakker statistiek korrellasies getoon. Goeie korrellasies is verkry tydens kalibrasie vir die C18:2/C16:0 en C18:2/C18:0 verhoudings.

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"The most perfect guide is nature.
Continue without fail to draw from it every day."

Ghirlandio, painter & teacher of
Michalangelo Buonarotti

CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters had, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

Chapter 1

INTRODUCTION

The continuous increase in world population has necessitated the development of new food resources for the fulfilment of the basic needs of the human race. An indispensable resource that has been undoubtedly indicated is the edible fats and oils sector. The increasing demand for edible fats and oils, has led to the nutritional and toxicological evaluation of oils obtained from unconventional sources and traditionally believed agricultural waste products. These products include rice-bran, cleome viscose, mahua, kapok, neem oil and mango kernel fat (Polasa & Rukmini, 1987). Mango kernel fat, derived from the kernel of the mango stone (with a fat content of 7.3 – 14.4%) (Puravankara *et al.*, 2000) has consequently raised much expectation in the vegetable oil world (Arogba, 1999).

The mango (*Mangifera indica* L.) is the most important fruit in Asia and currently ranks fifth in total production among major fruit crops worldwide (Hammerschlag & Litz, 1997). More than 80% of the world mango production is annually obtained from Africa and Asia (Teguia, 1995). It is grown naturally or cultivated mainly in tropical and subtropical regions. Mango species have, however, been grown in very dry areas, on permanently or temporarily inundated lands, at altitudes over 1000 m in the tropics, at higher latitudes, outside the tropics, under monsoon climate conditions and even under ever wet conditions, while still producing a good crop (Kostermans & Bompard, 1993). After consumption or industrial processing of the fruit, considerable amounts of mango stones are discarded as waste (Puravankara *et al.*, 1999; South African Mango Growers Association, 2002).

The stone of the mango fruit consists of a kernel enclosed by a rather woody to leathery endocarp with longitudinal curved incised lines (Kostermans & Bompard, 1993). As several cultivars of mango are grown, the size, shape and weight of mango stones, kernels and their fat content vary from as high as 23% to as low as 9% on the weight of the fruit (Narasimha Char & Azeemoddin, 1988; Palaniswamy *et al.*, 1974). The kernels contain on average 5.7% protein, 9.3% fat, 79.9% carbohydrate, 2.0% crude fibre and 3.1% ash.

The ranges of fatty acids present in the kernel of *Mangifera indica* L. have been reported as palmitic (C16:0; 3 – 18%), stearic (C18:0; 24 – 57%), oleic (C18;

34 – 56%), linoleic (C18:2; 1 – 13%) and arachidic (C20:0; traces) (Lakshiminarayana *et al.*, 1983). Trace amounts of C18:3 fatty acid have also been reported (Van Pee *et al.*, 1980). The nitrogen compounds consist of albumin, globulins, prolamines, gluteline and non-protein nitrogen. The starch content is 70.76% and the main free sugars are glucose and fructose. Significant amounts of calcium, copper and magnesium were found (Zazueta-Morales *et al.,* 1999). No indication of tocopherol content was found, but Kabuki *et al.* (2000) confirmed the presence of polyphenols in mango kernel extract (MKE), measuring the total polyphenol content as 79.5%.

The oxidative stability of edible fats and oils is of the utmost importance for the producer and consumer, as oxidative deterioration is responsible for the production of odorous compounds as well as the decrease in nutritional quality of the final product (Nawar, 1996). It is therefore necessary to monitor the oxidative deterioration of fats and oils and if possible employ methods to slow oxidation as far as possible (Maloba *et al.*, 1996). The two main factors influencing the shelf life of fats and oils are heat and oxygen (Gunstone, 1996).

From the view of the chemical properties of fats and oils, the important lipids are those containing unsaturated fatty acids, particularly oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) (White, 1995). The susceptibility and rate of oxidation of these fatty acids increase in relation to their degree of unsaturation (Gunstone, 1996). The greater the number of double bonds, the greater the probability that the fatty acid will react with oxygen to generate undesirable flavours in the product (Marsili, 1993). Knowledge of the fatty acid profile is thus very important when analysing the oxidative stability of fats and oils.

Tests for monitoring the oxidative status of oils include the peroxide value, iodine value, conjugated diene value, free fatty acids, *p*-anisidine value and thiobarbituric acid value tests. Oxidative deterioration of lipids can also be determined by measuring the changes in fatty acids occurring over a period of time. This can be executed using various chromatographic methods, including gas chromatography, thin layer chromatography and high performance liquid chromatography.

No references were found concerning the oxidative stability of MKF and the only indication of oxidative stability could be made in view of the fatty acid profile obtained from literature. The aim of this study was to determine the oxidative

stability of crude, cold pressed mango kernel fat produced in South Africa. The objectives were the optimisation of a method for the determination of long chain fatty acids in mango kernel fat, the monitoring of primary (peroxide value & conjugated diene value tests) and secondary oxidation (*p*-anisidine value) over time and the development of a Fourier transform near infrared calibration model to predict the oxidative status of mango kernel fat.

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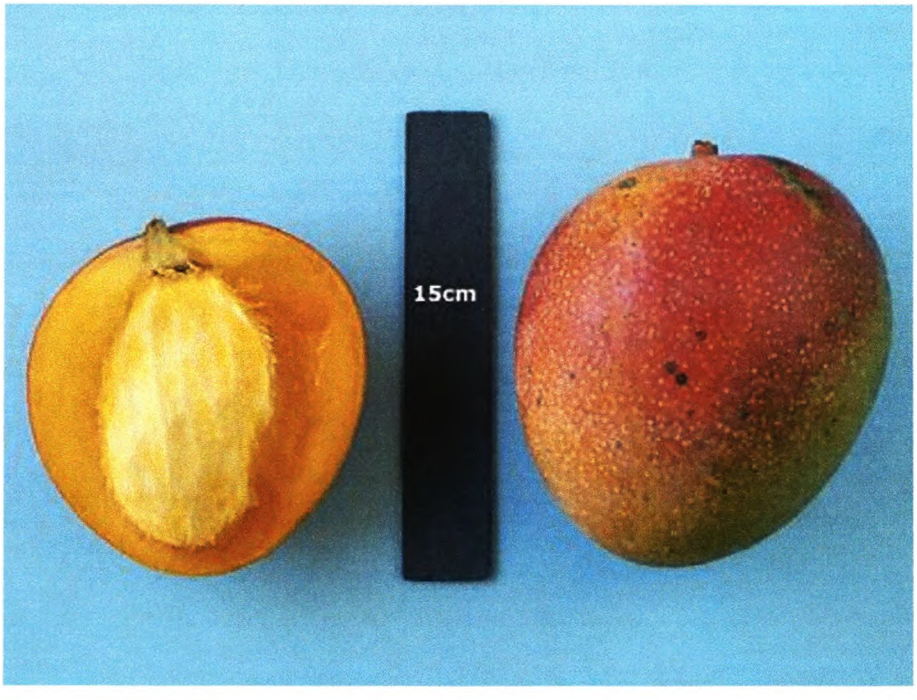
CHAPTER 2

LITERATURE REVIEW



Chapter 2

LITERATURE REVIEW

A Introduction

The use of animal and vegetable fats and oils for edible purposes in everyday life has a history as long as mankind itself, dating back to ancient cultures such as the Chinese, Egyptian and Greco-Roman (Hoffmann, 1989). The sources of these lipids were, as they are now, plants and animals, the latter being primarily land animals, with the use of marine animals being secondary (Nawar, 1996). From the oldest known fats, which were found in pots in the tombs of pharaohs, very little has changed concerning the importance of this substance for household and industrial purposes (Hoffmann, 1989).

In the current health conscious world, fat has become a misinterpreted term and has been at the centre of controversy with respect to toxicity, obesity and disease (Nawar, 1996). Due to a lack of knowledge, many of the related contentions and claims concerning fats are false. All fats have a unique fatty acid profile and a common misconception held by the public is that all fats are essentially the same (Byrnes, 2001).

Dietary fats of animal or vegetable origin are classified as *visible* (adipose tissue, milk fat, seed oils) or *invisible* (derived from animal or vegetable membranes) (Byrnes, 2001). Oils and fats, consist mainly of triacylglycerols as well as minor amounts of phospholipids, glycolipids, sterol esters and vitamins (Horton *et al.*, 1996). Fats are the richest source of energy on a weight basis and excess fat beyond the daily energy requirement is laid down as reserve depot fat, usually after some structural modification (Gunstone, 1996).

Dietary fat is the necessary source of essential fatty acids (EFAs) of the *n*-6 and *n*-3 types (Gunstone, 1996). These fatty acids are referred to by designating double bond positions with respect to the end CH_3-group with symbols such as $\omega 6$ or *n*-6, which indicate that the first double bond is on carbon 6, counting from the methyl group (Gunstone, 1996). The two EFAs, linoleic (omega-6 fatty acid) and γ-linolenic (omega-3 fatty acid), are needed for the maintenance of biomembrane integrity and for the metabolism of the various products of the eicosanoid cascade (Garret & Grisham, 1995), while α-linolenic acid is not absolutely required by animal organisms

(Hoffmann, 1989). Nutritionally, arachidonic acid is also considered as an essential fatty acid, even though it is assumed to be synthesised *in vivo* from linoleic acid (Namiki, 1995). Some dietary fats are also required sources of the fat-soluble vitamins, i.e. A, D, E, and K (Horton *et al.*, 1996).

Typical commercial sources of EFAs are palm and palm kernel oil, as well as coconut, soybean, rape, sunflower (Gunstone, 1996), cottonseed, peanut and olive oil (Nawar, 1996). In poor countries with deficient supplies of these sources, a sufficient substitute is a necessity (Narasimha Char & Azeemoddin, 1988) and the pressure of an ever-growing world population has led to the motivation for the development of new sources of fats and oils (Hoffmann, 1989). In view of the shortage of edible fats and oils in developing countries, nutritional and toxicological evaluations have been carried out on some unconventional oils to determine whether they might be safe for human consumption (Polasa & Rukmini, 1987). These lipids include rice-bran oil, cleome viscose oil, mahua oil, kapok oil, neem oil and mango kernel fat. Attention has been given especially to the utilisation of traditionally accepted agricultural waste products (Narasimha Char & Azeemoddin, 1988). One of these agricultural waste products with immense potential as a source of edible fat is the stone (or seed) derived from the mango fruit (Puravankara *et al.*, 2000).

After consumption or industrial processing of mango fruit, considerable amounts of mango stones are discarded as waste. Approximately 3×10^5 tonnes of dry kernels from these mango stones are available annually in India (Puravankara *et al.*, 2000; Narasimha Char & Azeemoddin, 1988). The fact that the mango kernel contains an average of approximately 7.3 – 14.4% fat has raised hopes in the vegetable oil world (Arogba, 1999). The development of a feasible technology for the exploitation of waste mango stone has assumed a noteworthy significance, as recovery of this fat is not only a technological challenge, but an economic inevitability too (Narasimha Char & Azeemoddin, 1988).

B Fats and oils as commercial commodities

In the 1991/1992 harvest year, the annual world production of oils and fats was 83.7 million tonnes, of which about 23.7 million tonnes (31%) entered into world exports, with the remainder being used in the country where it was produced (Gunstone, 1996). Three-quarters of the total production was of vegetable origin. Eighty percent of this material was used for human food and a further 6% for animal feed to produce

yet more human food. About 14% (*ca.* 12 million tonnes) served as source material for the oleochemical industry. About 90% of this was used in the production of soap and other surface-active compounds and the balance, representing about 1.2 million tonnes, was used for other industrial purposes.

The world prodution of major oils is expected to increase almost four-fold from 1965 to 2005. Oil production is estimated to reach 115 million tonnes in 2005 (Gunstone, 1996). This can be compared with the annual production of mineral oil and coal (3000 million tonnes each), wheat and rice (*ca.* 400 million tonnes each), and potatoes (*ca.* 300 million tonnes) in the 1991/1992 harvest year.

The most significant oils are palm, palm kernel, coconut, soybean, rape and sunflower oil (Gunstone, 1996). The proportion of these six oils represented 33% of total oil and fat production in 1965, rose to 55% by 1985 and is expected to be 63% in 2005. Oils with high percentages of C16 and C17, as well as lauric (C12:0) acid oils, show the greatest growth in the oils and fats sector.

The demand for oils and fats for food and non-food use is linked particularly with the growing world population and with income growth. In 1991/1992 the average annual usage of fats for both food and non-food uses was 15.3 kg per person (Gunstone, 1996). This covers a range of values, from 35 to 40 kg in most affluent countries (North America, Western Europe and Australasia), about 7 kg in the highly populated countries of India and China, to even lower levels in many of the poorer countries. In view of the very large populations in India and China, the global demand for oils and fats is likely to grow for some time (Gunstone, 1996).

Although those living in affluent countries are encouraged to reduce their total fat intake and to have the appropriate balance of saturated, monounsaturated and *n*-6 and *n*-3 polyunsaturated fatty acids, there are heavily populated communities whose main requirement is for calories, which are most efficiently provided by fats of any kind (Byrnes, 2001).

C Mango kernel fat

C1 Background

The mango (*Mangifera indica* L.) is the most important fruit in Asia and currently ranks fifth in total production among major fruit crops worldwide (Hammerschlag & Litz, 1997). It is grown either naturally or cultivated, mainly in tropical and subtropical regions. Mango species, however, have been grown in very dry areas, on

permanently or temporarily inundated lands, at altitudes over 1000 m in the tropics, at higher latitudes, outside the tropics, under monsoon climate conditions and even under ever wet conditions, while still producing a good crop (Kostermans & Bompard, 1993).

The stone of the mango fruit consists of a kernel enclosed by a rather woody to leathery endocarp with longitudinal curved incised lines (Kostermans & Bompard, 1993). As several cultivars of mango are grown, the size, shape and weight of mango stones, kernels and their fat content vary widely (Narasimha Char & Azeemoddin, 1988). The stone content varies between cultivars, ranging from as high as 23% to as low as 9% of the weight of the fruit (Palaniswamy *et al.*, 1974). The kernels, depending on the variety, contain on average 5.7% protein, 9.3% fat, 79.9% carbohydrate, 2.0% crude fibre and 3.11% ash. In times of scarcity and famine, mango seed kernels are consumed by the poor after they have been boiled (Puravankara *et al.*, 2000).

C2 Mango kernel

Large quantities of mango fruit are produced in Africa and Asia (0.5 to 1.5 tonnes.ha^{-1}, depending on cultivar and planting density). More than 80% of the world mango production recorded annually is obtained from these continents (Teguia, 1995). An estimated 10 million tonnes of mango fruit is produced in India every year, producing one million tonnes of mango stones (Narasimha Char & Azeemoddin, 1988). From these stones, 0.3 million tonnes of kernel could be obtained. About 30 000 tonnes of mango kernel fat, valued at 450 million Indian rupees (*ca.* R100 million), could be recovered from this waste mango seed if the kernels are collected and used properly (Narasimha Char & Azeemoddin, 1988; Rukmini & Vijayaraghavan, 1984).

In Egypt, many varieties are grown in large quantities, yielding 50 000 tonnes of fruit annually (Amin & El Sayed, 1973). South Africa produced 67 348 tonnes of fruit in the 2000/2001 harvest year and 87 583 tonnes in the 2001/2002 harvest year (South African Mango Growers' Association, 2002). After consumption or industrial processing of the fruit, considerable amounts of mango seeds are discarded as waste globally (Puravankara *et al.*, 2000; South African Mango Growers' Association, 2002). Excluding the informal sector, approximately 2340 tonnes of mango kernel is

available annually in South Africa (South African Mango Growers' Association, 2002).

The mango kernel is potentially a source of edible fat and since a vegetable oil famine is currently a reality in India and other developing countries, this generous amount of edible grade fat, recovered from a cheap and neglected agro-waste material, can hardly be ignored (Narasimha Char & Azeemoddin, 1988).

A study conducted by Lásztity *et al.* (1988) investigated the gross chemical composition, protein content and amino acid composition of the mango stone kernels of four Egyptian varieties (Eswi, Henndi, Fonso and Timour). A high glutamic acid, aspartic acid and leucine content and a low concentration of sulfur-containing amino acids were detected. Starch has also been isolated from the mango kernel and its physical properties have been studied (Amin & El Sayed, 1973). The utilisation of this starch is evident as the kernels are used for feeding mammals and chickens.

The steps involved in the processing of mango stone for fat recovery are collection, cleaning and drying, decortication of mango stone to release the kernel and pressing or solvent extraction (Narasimha Char & Azeemoddin, 1988). Mango stones can be collected by manual labour from under the trees or from fruit industries as a by-product, with the latter being the cleaner and healthier option (Narasimha Char & Azeemoddin, 1988). Decortication is done either by hand with a knife or with the modern mango stone decorticators, which are considered an important breakthrough in the utilisation of mango stones (Narasima Char & Azeemoddin, 1988). The assorted odd sizes, shapes and toughness of the mango stones make it difficult to decorticate and remove the inner kernel from the bivalve shells. Narasimha Char *et al.* (1975) developed a mechanical device for decortication with a high efficiency on both wet, fresh, dry and old mango stones.The high moisture content of the kernel makes it prone to rapid spoilage and ill-kept surroundings hasten the process of deterioration (Narasimha Char & Azeemoddin, 1988).

Narasimha Char *et al.* (1979) developed a low-cost hand-operated rotary dryer for drying freshly collected mango stones and mango kernels. Dried mango stones and kernels, on the other hand, can be stored without problems for a period of 120 days without any damage in either quantity or quality of the fat present in the kernel.
After decortication, the extraction of the fat from the kernel is the most important step in the processing of mango kernels. Narasimha Char *et al.* (1977) have carried out several investigations and developed easily adoptable methods to extract fat from the

kernels and purify crude mango kernel fat for the preparation of edible grade refined fat for human consumption. In one of the methods reported, the kernels were separated after decortication, cooked and passed through an expeller after which the resulting cake was extracted with hexane. The crude fat obtained was refined and bleached using AOCS methods (Narasimha Char *et al.*, 1977; Narasimha Char & Azeemoddin, 1988). The method used in South Africa by Specialised Oil cc. includes collection of the mango stones, decortication, drying and mechanical extraction of the fat (Specialised Oil cc., 2002).

Mango kernel fat closely resembles cocoa butter in its physical and chemical characteristics, thereby ensuring its importance in international markets (Palaniswamy *et al.*, 1974). According to EU legislation, six kinds of vegetable fat are permitted in chocolate production in addition to cocoa butter, i.e. sal butter, mango kernel fat, kokum fat, illipe butter, shea butter and palm oil (Anonymous, 2000).

C3 Chemical composition

Mangifera indica L. belongs to those taxa that have starch-rich seeds, of which the kernels represent approximately 50% of the whole seeds and contain 9-13% fat and 50-57% starch (Kostermans & Bompard, 1993).

Due to its fatty acid arrangement, mango kernel fat can be described as a vegetable butter (Gunstone, 1996). "Butters" melt over a relatively narrow range of temperatures because their triacylglycerols are mainly palmitic-oleic-stearic, stearic-oleic-stearic and palmitic-oleic-palmitic (Nawar, 1996). Fats from this group are derived from the seeds of various tropical trees and are used extensively in the manufacture of confectionery, with cocoa butter being the most important member of the group (Gunstone, 1996). A comparison of the characteristics, fatty acids and chemical compositions of cocoa butter and mango kernel fat indicates the similarities in chemical and physical characteristics (Tables 2.1 & 2.2). The fatty acid composition of the two fats is closely related. Mango kernel fat is characterised by large amounts of saturated fatty acids and contains tristearin and stearopalmitostearin, which are triacylglycerides that are rather rare in plants.

The nitrogen composition (Table 2.3) consists of albumin, globulins, prolamines, gluteline and non-protein nitrogen. The starch content is 70.76% and the main free sugars are glucose and fructose.

Table 2.1 Some physicochemical characteristics of mango kernel fat and cocoa butter
(Narasimha Char & Azeemoddin, 1988; Anonymous, 2001)

Characteristics	Mango kernel fat	Cocoa butter
Melting point (°C)	34 - 43	34.5
Slip point (°C)	30	32
Acid value	2 - 6	---
Saponification value	193 - 195	196
Iodine value (Wijs)	32 - 57	37.6
Colour	Pale yellow	Pale yellow

Table 2.2 Fatty acid composition of mango kernel fat and cocoa butter (Lakshiminarayana *et al.*, 1983; Narasimha Char & Azeemoddin, 1988;)

Fatty acid	Mango kernel fat	Cocoa butter
16:0 (Palmitic)	6 – 18%	24.3 - 27.0%
18:0 (Stearic)	24 – 57%	35.4 - 37.5%
18:1 (Oleic)	33 - 56%	33.6 - 38.1%
18:2 (Linoleic)	1 – 13%	2.2 - 2.5%
20:0 (Eicosanoic)	1 - 2.6%	-

Table 2.3 The protein, starch & sugar content of mango kernel (Zazueta-Morales *et al.*, 1999)

Constituent	Content (%)
Protein	
Albumin, globulins	9.95
Prolamines, gluteline	73.55
Non-protein nitrogen	18.16
Starch	70.76
Sugars	
Glucose	0.1
Fructose	0.1

Significant amounts of calcium, copper and magnesium were found (Zazueta-Morales *et al.,* 1999). Average values for the characteristics of mango kernels are depicted in Table 2.4.

C4 Anti–microbial action

Kabuki *et al.* (2000) reported the presence of anti-microbial activity against food-borne pathogenic bacteria in the ethanol extract of mango seed kernel. The mango kernel extract showed an anti-microbial activity against both gram-positive and gram-negative bacteria, with the latter being less active (Kabuki *et al.*, 2000). The anti-microbial peptide, nisin, produced by *Lactococci lactis*, has a similar anti-microbial spectrum to the mango kernel extract.

Mango kernel extract is heat and pH stable and can be applied in a variety of foods (Kabuki *et al.*, 2000). Although more precise toxicity studies are needed, the mango kernel seems to be a promising additive for extending the shelf life of a variety of food products (Kabuki *et al.*, 2000).

C5 Polyphenols

Phenolic antioxidants are present in a wide range of plant sources and the use of herbs and spices to preserve foods predates the concept of shelf life (Gunstone, 1996). Flavonoids, which occur naturally in plants, are recognised as important compounds in conferring stability against autoxidation in vegetable oils (Das & Pereira, 1990). Their effectiveness in this regard is related to their ability to act as free-radical acceptors or chelators of metal ions.

A new source of polyphenols was discovered when Parmar & Sharma (1986) reported that mango seed kernel (containing 11.64% fat) enhanced the oxidative stability of ghee, a fresh-type cheese. Kabuki *et al.* (2000) confirmed the presence of polyphenols in mango kernel extract. Tannins and flavones were detected and the total polyphenol content was measured at 79.5%.

C6 Tocopherols

Vitamin E is a fat-soluble vitamin and is found at high concentrations in vegetable oils (Tawfik & Huyghebaert, 1997). Good vitamin E sources (i.e. soybean oil) are identified by high amounts of the α isomer, whereas effective antioxidants are

Table 2.4 Average values for characteristics of mango kernel (Zazueta-Morales *et al.*, 1999)

Characteristic	Value
Specific gravity	1.01
Bulk density	580 $kg.m^{-3}$
Dimensions	59 x 34 x 17 mm
Lipids	6.83%
Protein	7.93%
Crude fibre	69.19%
Ash	2.46%
Carbohydrates	73.09%
In vitro digestibility of starch	69.19%
Tannins	1.28 $mg.g^{-1}$
Phytic acid	1.73 $mg.g^{-1}$
Water/g dry solids	1.44 g
pH	4.5
Isoelectric point of proteins	5.0

characterised by high levels of the γ and δ isomers, as the α isomer has shown pro-oxidant activity at levels of 500 ppm. These isomers are found in varying concentrations in vegetable oils and find application according to their constituents (Table 2.5) (Gunstone, 1996). No references were found concerning the tocopherol content of mango kernel fat.

C7 Potential use

Nutritional and toxicological evaluations conducted on mango seed kernels have indicated their suitability as an ingredient in human and animal nutrition (Arogba, 1999; Teguia, 1995). Contradictory to this, Kabuki *et al.* (2000) suggested that more precise toxicity studies were needed in this regard. Due to its blandness, plasticity and absence of toxic substances, the kernel fat has potential use in the preparation of sweetmeats (Narasimha Char *et al.*, 1977) and the manufacturing of toffees (Moharram & Moustafa, 1982). It is also used as an extender fat in western countries, especially in baking, confectionery and as a substitute for cocoa butter (Baliga & Shitole, 1981). The incorporation of mango kernel into wheat and maize-supplementation programmes in developing countries has commercial potential and may result in products with a higher energy content and optimum protein supplementation (Joseph & Abolaji, 1997; Arogba, 1999).

In additional to its application in the food industry, mango kernel fat can be incorporated in the production of pharmaceutical products and in soap manufacturing (Narasimha Char & Azeemoddin, 1988). After extraction of the fat, the defatted mango kernel, which is rich in starch, may then find application in the textile, jute and paper industries and can also be used as good plant manure, ensuring that nothing goes to waste (Narasimha Char & Azeemoddin, 1988).

D Chemistry of fats and oils

D1 Fatty acids

Fatty acids are long, straight hydrocarbon chains with a carboxyl group attached to their end (Baggot & Dennis, 1997) (Figure 2.1). When three fatty acids are bonded together with a glycerol molecule, the result is a triacylglyceride (Garret & Grisham, 1995). Monoacylglycerols and diacylglycerols also exist, but are far less common than the triacylglycerols. Mixed triacylglycerols contain two or three different fatty

Table 2.5 Tocopherol levels in selected vegetable oils ($mg.100g^{-1}$) (Gunstone, 1996).

Oil source	Tocopherols			
	α	β	γ	δ
Palm	25.6	-	31.6	7
Rape	21.0	0.1	4.2	-
Soybean	7.5	1.5	79.7	26.6
Sunflower	48.7	-	5.1	0.8
Walnut	56.3	-	59.5	45.0
Wheatgerm	133	7.1	26.0	27.1

acids, while simple triacylglycerols contain identical fatty acids (Garret & Grisham, 1995). The many fatty acids, which occur naturally, arise primarily through variation in chain length and degree of saturation (Horton *et al.*, 1996). All fatty acids are classified according to the number of carbon atoms present in their structure, as well as the degree of saturation or how many hydrogen atoms are bonded to the carbons (Horton *et al.*, 1996). A fatty acid that has two hydrogen atoms linked to each carbon atom is saturated (Figure 2.1), while a fatty acid with two hydrogens absent is monounsaturated (Figure 2.2) and a fatty acid with four or more hydrogens absent is polyunsaturated (Baggot & Dennis, 1997) (Figure 2.3). All fats and oils, whether of animal or vegetable origin, are blends of these three types, but with one usually predominating, depending on the food in question (Byrnes, 2001). When saturated fatty acids dominate, the lipid will generally be referred to as a fat, while the domination of unsaturated fatty acids will characterise the lipid as an oil. Saturation of the fat is directly proportional to the chemical stability of the fat (Gunstone, 1996).

Typically, saturated fatty acids are waxy solids at room temperature (22°C), whereas unsaturated fatty acids are liquids at this temperature (Horton *et al.*, 1996). The melting point, boiling point and refractive index of saturated fats all increase with increasing chain length (Hoffmann, 1989). The higher melting point is due to the increasing Van der Waals interactions among neighbouring hydrocarbon chains, which require more energy to disrupt the interactions (Horton *et al.*, 1996). As the degree of unsaturation increases, fatty acids become more fluid. Palmitic acid (C16:0, melting point 63°C) and stearic acid (C18:0, melting point 70°C) are solid at body temperature, but oleic acid (C18:1, melting point 13°C) and linolenic acid (C18:3, melting point –17°C) are both liquids (Horton *et al.*, 1996).

Saturated fats predominate principally in animal fats, although palm and coconut oils and the "butters" obtained from tropical trees are noted plant sources of saturated fatty acids (Baggot & Dennis, 1997). Monounsaturated fats abound in nuts, avocados, olive oil and some animal fats (especially lard). Polyunsaturated fats are the main constituents of vegetable oils, but significant amounts are found in fish oils and chicken skin (Baggot & Dennis, 1997).

Although fatty acid synthesis takes place in the living organism, mammals require some essential polyunsaturated fatty acids that cannot be synthesised and must be acquired through their diet (Garret & Grisham, 1995). Although essential, the intake of EFAs should not exceed 5% of the total caloric intake (Seidelin *et al.*, 1992).

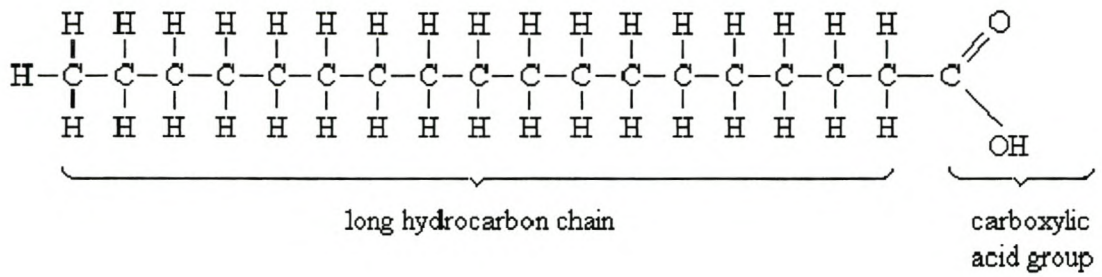


Figure 2.1 Stearic acid (C18:0), a saturated fatty acid.

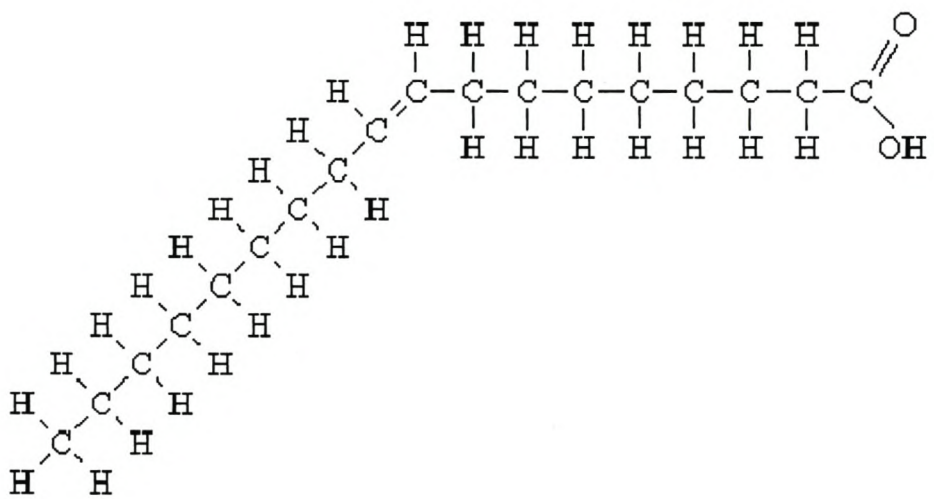


Figure 2.2 Oleic acid (C18:1), a monounsaturated fatty acid.

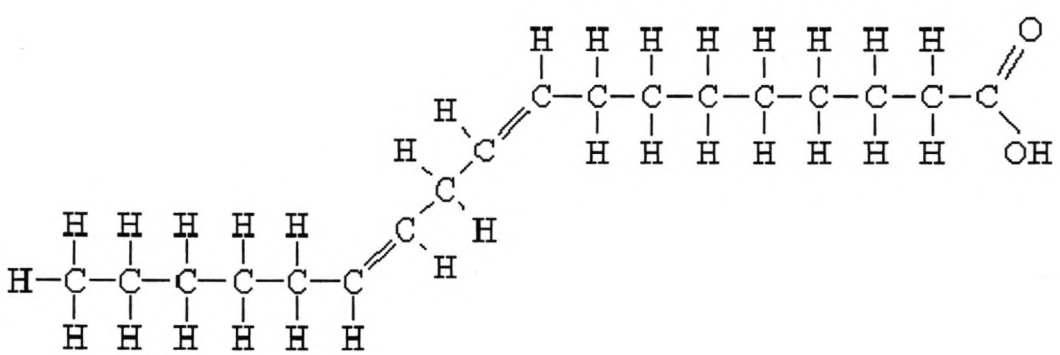


Figure 2.3 Linoleic acid (C18:2), a polyunsaturated fatty acid.

Essential fatty acids are utilised by the body to create other omega-3 and 6 fatty acids and hormone-like substances called prostaglandins, which carry out metabolic functions (Namiki, 1995). Prostaglandins produced from *n-3* fatty acids are different from those derived from *n*-6 fatty acids and have platelet anti-coagulative action. In this connection, it has been stated that oil with a low *n-3* fatty acid content is an inferior source of prostaglandin (Namiki, 1995).

Whilst omega-*3* fatty acids are found primarily in cold water fish, walnuts, eggs, flax oil, dark green leafy vegetables, cod liver oil and some whole grains, omega-6 fatty acids are found mainly in vegetables (Gunstone, 1996). When there is an overabundance of linoleic acid in the diet, the body's ability to absorb and utilise linolenic acid is inhibited (Seidelin *et al.*, 1992). This causes a host of undesirable reactions, including sexual and immune dysfunction and increased cancer risk. Due to the increased intake of vegetables and thus of linoleic acid in the Western world, the occurrence of cancer and heart disease has skyrocketed (Baggot & Dennis, 1997).

The other type of fatty acid, the trans-fatty acid, is produced during chemical processing. These unnatural fats cannot be utilised by the body because of their structure and are made by forcing hydrogen atoms into a liquid vegetable oil and producing the appearance of a saturated fat. It is the consumption of these trans-fatty acids, opposed to that of saturated fatty acids, that is strongly correlated with the occurrence of cancer, cardiovascular disease and other diseases (Gunstone, 1996).

D2 Lipid oxidation

Oxidative stability

The health trend is currently moving away from the use of saturated fats to oils containing high levels of monounsaturated and polyunsaturated fatty acids (Marsili, 1993). Although this may mean a healthier product for the consumer, it can also cause more problems for food processors, because of the increased potential for oxidation off-flavour development (Gunstone, 1996). From the view of lipid oxidation, the important lipids are those containing unsaturated fatty acids, particularly oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) (White, 1995).

The susceptibility to and rate of oxidation of these fatty acids increase in a somewhat geometric fashion in relation to their degree of unsaturation (Gunstone, 1996). The greater the number of double bonds, the greater the probability that the

fatty acid will react with oxygen to generate undesirable flavours in the product (Marsili, 1993).

A main source of off-flavours in many foods has traditionally been the reaction of lipid components with oxygen in the presence of light and/or heat through autoxidation, which is a self-catalytic mechanism (Nawar, 1996). When oxygen is unlimited, the oxidation rate depends on the relation between oil surface area exposed to air and sample volume (Crapiste *et al.*, 1999), but when the oxygen concentration is low, the rate is approximately proportional to oxygen concentration (Nawar, 1996).

Overheating of oils during processing in the industry is a major way in which oil hydrolysis can occur (Marsili, 1993), causing the production of oxidation products that can impart highly objectionable aromas and flavours, ranging from the odour of "sweaty socks" to "soapy" tastes. In addition, oxidative reactions can decrease the nutritional quality of food and certain oxidation products are potentially toxic (Nawar, 1996). Lipid oxidation is of great concern to the food manufacturer and the methods that have been implemented to slow down oxidation include chilled storage, freezing, drying, modified-atmosphere packaging and the use of chemical additives and preservatives, including antioxidants (Maloba *et al.*, 1996).

Since no simple parameter provides enough information for a correct assessment of oxidation, it is necessary either to perform several analyses or to determine the composition of polar compounds in order to evaluate different stages of oxidation (Crapiste *et al.*, 1999).

D3 Mechanism of lipid oxidation

Hydroperoxides, generally referred to as peroxides, are the major initial reaction products of unsaturated fatty acids with oxygen (McClements, 2000). These compounds are highly reactive reaction intermediates and may be directly responsible for the off-flavour development of lipid oxidation aromas (Marsili, 1993) by causing the formation of alkoxyl radicals ($RO^{\bullet}$), which leads to *b*-scission reactions, which in turn lead to the formation of a variety of odorous molecules, including aldehydes, ketones and alcohols (McClements, 2000).

Many volatile components that are formed from the decomposition of hydroperoxides of unsaturated fatty acids in oils or fats have been identified (Shierberle & Grosch, 1989). In some cases, a relationship has been established

between their quantity and flavour properties. Depending on the number of double bonds and the oxidation mechanism involved, each fatty acid produces different hydroperoxides, which eventually leads to the formation of a wide variety of volatiles and, consequently, diverse odours (Gunstone, 1996).

Lipid oxidation can be divided into three steps: Initiation, propagation and termination (McClements, 2000). During initiation, highly reactive free radicals are created when oxygen reacts with a substrate (fatty acids) (reaction equations 1 & 2). These unpaired electrons are highly reactive with a short lifespan in their search for another unpaired electron (Gunstone, 1996). The initiation step cannot be stopped by additives. Only the exclusion of radical formers can inhibit free radical production (Hoffmann, 1989). Singlet oxygen is the active species involved in catalysing the production of the first free radicals necessary to start the oxidation process (Nawar, 1996).

Initiation $O_2 + RH \rightarrow R^{\bullet} + HOO^{\bullet}$ **...1**

The rate of oxygen absorption, which was determined experimentally (mostly with ethyl linoleate) can be calculated (Equation A) (Nawar, 1996):

$$Rate = -\frac{d[O_2]}{dt} = \frac{K_a[RH][ROOH]}{1 + \lambda[RH]/p} \qquad \textbf{...A}$$

Where:
- RH = Substrate fatty acid
- H = α-methylenic hydrogen atom (easily detachable due to the influence of a neighbouring doule bond or bonds)
- ROOH = Hydroperoxide formed
- p = Oxygen pressure
- λ and K_a = Empirical constants

During propagation, atmospheric oxygen reacts with the free radicals to form peroxy radicals ($ROO^{\bullet}$) (reaction equation 2). Secondly, these highly reactive free radicals

react with other unsaturated fatty acids to form hydroperoxides (ROOH) (reaction equation 3), which are odourless and tasteless (Hoffmann, 1989). This second step is also the stage where a number of protective measures can be taken (Hoffmann, 1989).

These free radicals can repeat the process, but the hydroperoxides are also broken down to form free radicals and a variety of compounds with a lower molecular weight (Nawar, 1996) (reaction equation 4). These compounds are mainly aldehydes, which are largely responsible for the development of the undesirable taste and flavour, characteristically of rancid fat (Gunstone, 1996). These typical odours are often described as green, beany, painty, fishy, tallowy or just rancid odours (Hoffmann, 1989).

Propagation	$R^{\bullet} + O_2$	→	$ROO^{\bullet}$	**...2**
	$ROO^{\bullet} + RH$	→	$ROOH + R^{\bullet}$	**...3**
	$2ROOH$	→	$RO^{\bullet} + ROO^{\bullet} + H_2O$	**...4**

The increase in free radicals leads to radicals reacting with each other to form stable end products. This step is known as termination (Equation 5) (Nawar, 1996).

Termination	$R^{\bullet}, RO^{\bullet}, ROO^{\bullet}$	→	Stable Species	**...5**

As a rule, the radical reactions do not continue until total consumption of the fat substrate, because there usually is a depletion of oxygen supply. Oxygen transfer is inhibited by a (polymerised) film formed in the course of the reaction at the surface of the bulk of the oil or fat (Hoffmann, 1989). The high viscosity of used oils can be explained by polymerisation of the free radicals (Nawar, 1996).

Factors influencing oxidation

Oxygen concentration

Oxygen is about three times more soluble in oils than in water, with the result that there is always likely to be sufficient oxygen present in the oil phase to fuel lipid oxidation, unless specific measures are taken to exclude it (Nawar, 1996).

The orbital picture of oxygen indicates that there are two ways in which electrons can be inserted, as this molecule behaves like a biradical (Ulberth, 1997). It is unlikely that initiation occurs by the direct attack of oxygen in its most stable form (triplet state) on the double bonds of fatty acids (RH) (Nawar, 1996). Two unpaired electrons in the outer orbital can align their spins parallel or anti-parallel to each other, giving rise to two different multiplicities of state, i.e., $2(½+½)+1=3$ and $2(½-½)+1=1$. These are called triplet ($^3O_2$) and singlet state ($^1O_2$), respectively (Nawar, 1996). In the triplet state, the two electrons in the antibonding 2p orbitals have the same spin, but are kept apart by "Pauli exclusion", causing them to have a small repulsive electrostatic energy; while singlet oxygen, with opposite spinning electrons, is in an excited state. Triplet oxygen is a diradical molecule and only reacts with radical compounds by a free radical mechanism, as shown in Figure 2.4 (Hahm & Min, 1995). Singlet oxygen on the other hand is a nonradical and electrophilic molecule and can react directly with nonradical, electron-rich, double bond-containing compounds, such as oleic, linoleic, and linolenic acids, by the nonradical mechanism shown in Figure 2.5.

Chemical structure of lipids

Ultimately, it is the chemical structure of a lipid molecule that determines its susceptibility to oxidation, particularly the number and location of the double bonds. Saturated lipids are considerably more stable to lipid oxidation than unsaturated lipids (White, 1995). In bulk lipids, the rate of oxidation of fatty acids increases as their degree of unsaturation increases (Gunstone, 1996). Thus, fats that contain high concentrations of polyunsaturated fatty acids are particularly prone to lipid oxidation. Relative rates of oxidation for arachidonic (C20:0), linolenic (C18:3), linoleic (C18:2) and oleic (C18:1) acids are approximately 40:20:10:1 (Nawar, 1996). The unstable fatty acids occurring in edible oils are linoleic (C18:2) and linolenic (C18:3) acids.

Oleic acid, however, shows a very stable nature against oxidation and has even been incorporated to increase the stability of vegetable oils (Frankel & Huang, 1994). The 1,4-pentadiene structure in linoleates makes them much more susceptible to oxidation than the propene system of oleate (Nawar, 1996). The methylene group at position 11 is doubly activated by the two adjacent double bonds.

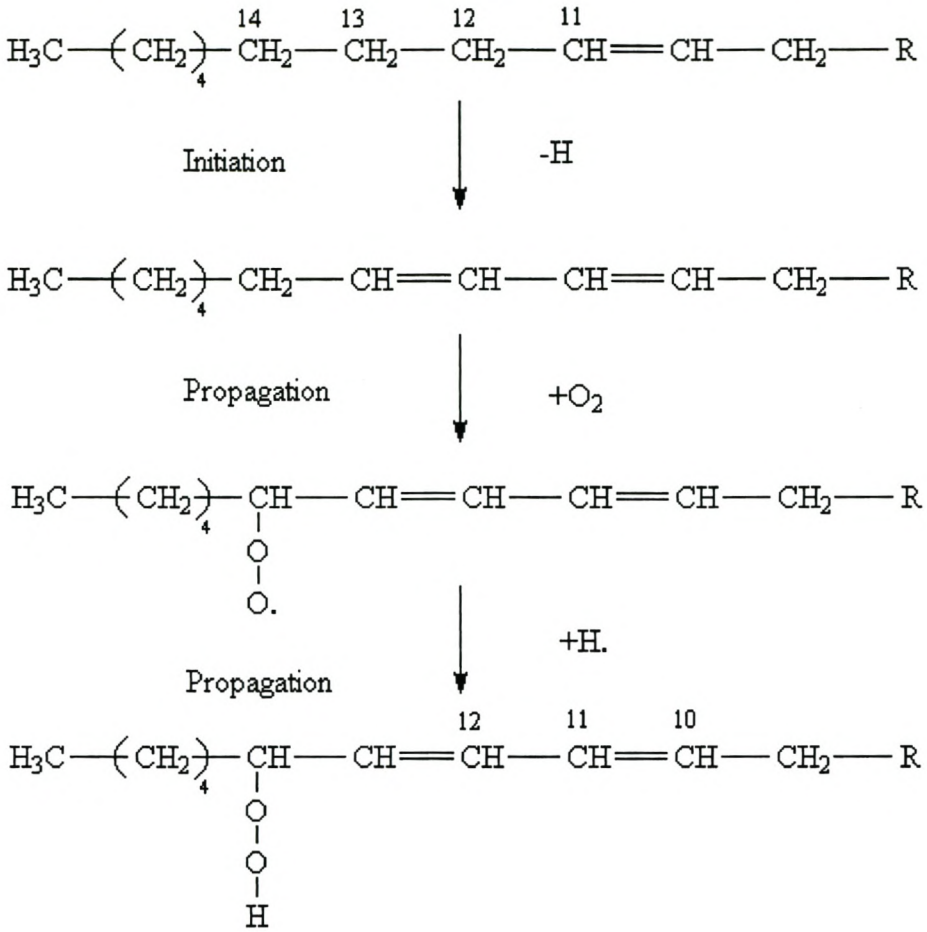


Figure 2.4 Formation of hydroperoxides of linoleic acid with triplet oxyen (Nawar, 1996).

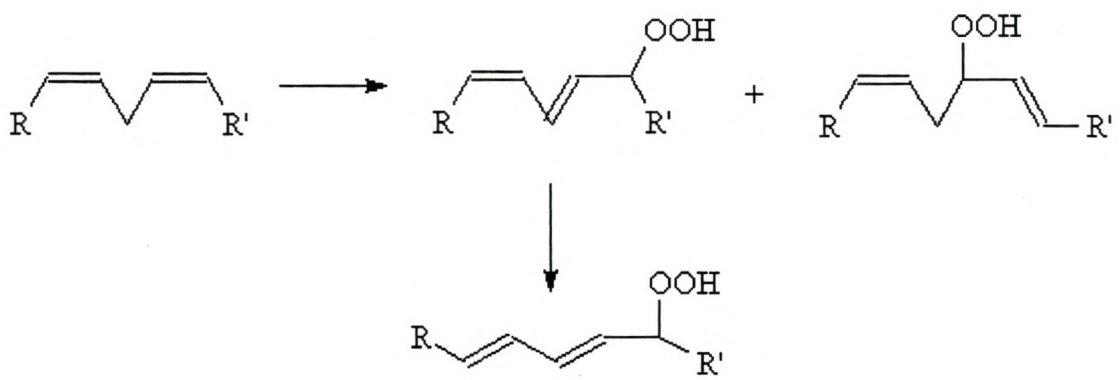


Figure 2.5 Formation of hydroperoxides of linoleic acid with singlet oxygen (Nawar, 1996).

A pentadienyl radical intermediate is produced at this position due to hydrogen abstraction, which produces an equal mixture of conjugated 9-and 13-diene hydroperoxides upon reaction with molecular oxygen. The position and geometry of the double bond on an unsaturated fatty acid will therefore especially influence its susceptibility to lipid oxidation (Baggot & Dennis, 1997). The closer the double bond is to the methyl end of a fatty acid, the greater its stability to oxidation. *Cis* acids oxidise more readily than their *trans* isomers, and conjugated double bonds are less reactive than the non-conjugated form (Nawar, 1996).

Packaging

Glass, metals and different kinds of plastic films are used for packaging of vegetable oils (Tawfik & Huyghebaert, 1997). Storage stability and shelf life for fats and oils are receiving attention among nutritionists, food processors, government regulators and consumers. The quality and shelf life of the packaged food are determined mainly by the barrier properties of the package against moisture, oxygen, light and the interaction of food constituents with the packaging materials (Ulberth, 1997).

When certain reactions occur spontaneously without external agents, packaging does not affect stability. In most cases, however, the environmentally omnipresent gaseous reactants, water vapour and oxygen, can seriously restrict stability under normal food storage and distribution conditions. Typically, these gases are not excluded effectively by unmodified plastic containers and, traditionally, glass containers are preferred.

Methods that have been used to slow or inhibit oxidation include the use of modified atmosphere packaging (MAP), in which the oil is stored in a gas-barrier material and the gaseous environment is changed so as to retard oxidation (Maloba *et al.*, 1996). The functions of this packaging include the oxidation of iron, incorporation of reducing agents, incorporation of antioxidants, autoxidation of the polymer by ground-state oxygen and conversion of triplet to singlet oxygen, whereafter it is absorbed. The use of amber packaging also positively influenced oxidative stability by excluding visible, ultraviolet and γ-radiation, which are effective promoters of oxidation (Tawfik & Huyghebaert, 1997).

Metal ions

Transition metals, particularly those possessing two or more valency states and a suitable oxidation-reduction potential between them and the lipid (e.g. cobalt, copper, iron, manganese and nickel), are effective pro-oxidants (Nawar, 1996). They can decrease the induction period and increase the rate of oxidation, even at concentrations as low as 0.1 ppm. Trace amounts of heavy metals are commonly encountered in edible oils, as they originate from the soil in which the oil-bearing plant was grown or from the metallic equipment used in processing or storage (Nawar, 1996).

Hydroperoxides are broken down by metal ions (Ulberth, 1997). An example of this is the interaction between of Fe^{2+} and hydroperoxides. This interaction between Fe^{2+} and hydroperoxides produces Fe^{3+} and an alkoxide free radical, but the Fe^{3+} is also capable of decomposing the hydroperoxide, giving a peroxy free radical and itself being reduced to Fe^{2+}. Large quantities of derived lipid are formed as a result of this reaction (Ulberth, 1997).

Moisture

The rate of oxidation depends strongly on water activity (a_w) in model lipid systems and in various fat-containing foods (Nawar, 1996). In dried foods with very low moisture contents (a_w values of less than 0.1), oxidation proceeds very rapidly. Increasing the a_w to about 0.3 retards lipid oxidation and often produces a minimum rate. This protective effect of small amounts of water is believed to occur by reducing the catalytic activity of metal catalysts by quenching free radicals, and/or by impeding the access of oxygen to the lipid (Nawar, 1996). At somewhat higher water activities ($a_w = 0.55 - 0.58$), the rate of oxidation increases again, presumably as a result of the increased mobilisation of catalysts and oxygen.

Antioxidants and vitamin E

A primary antioxidant ("chain-breaking-antioxidant") is a substance that is capable of accepting free radicals so that it can delay the initiation step or interrupt the propagation step of autoxidation by reacting with lipid and peroxyl radicals and converting them to more stable radical or nonradical products (McClements & Decker, 2001). Secondary antioxidants can retard lipid oxidation through a variety of mechanisms, including chelation of transition metals, replenishing of hydrogen to

primary antioxidants, oxygen scavenging, and deactivation of reactive species (Reische *et al.*, 1998). The main purpose of using antioxidants in lipids is to delay a significant accumulation of free radicals and thus to improve oxidative stability (Wanasundara & Shahidi, 1993).

Vegetable oils generally contain natural antioxidants, which are extracted along with the oil, though their level may be reduced during refinement (Gunstone, 1996). These compounds are usually tocols, i.e. tocopherols and tocotrienols. Tocopherols show two valuable properties, i.e. they have vitamin activity and are powerful natural antioxidants (Gunstone, 1996). These properties, however, are not identical. For vitamin E activity, the order is α (1.0)>β (0.5)>γ (0.1)>δ (0.03) and activity is usually expressed in α-tocopherol equivalents. For antioxidants this order is reversed (Gunstone, 1996). The antioxidative activity of tocopherols is $\gamma > \alpha$ *in vitro*, but the efficacy of γ-tocopherol in physiological vitamin E action is less than one-tenth of α-tocopherol (Namiki, 1995). Like other antioxidants, the tocopherols are themselves readily oxidisable (Koskas *et al.*, 1984). Mild oxidation of a tocopherol opens the heterocyclic ring to form tocoquinone, which is not an antioxidant.

Vitamin E is a fat-soluble vitamin and is found at high concentrations in vegetable oils (Tawfik & Huyghebaert, 1997). Therefore, palm oil for example, is a good source of vitamin E because of the high level of the α isomer, whereas the soybean tocopherols are effective antioxidants by virtue of the high levels of γ and δ isomers (Gunstone, 1996). The effectiveness of the tocotrienols is less fully understood (Gunstone, 1996). A study conducted by Wagner & Elmafda (2000) was based on the enrichment of olive and linseed oil with α-,γ- and δ-tocopherol in various concentrations. Oxidative stability was prolonged with higher additions of γ-tocopherol for both olive and linseed oil, although the stability decreased for linseed oil when the γ-tocopherol was raised above 158 mg.100 g^{-1} oil. For both olive and linseed oil, δ-tocopherol was more effective in the prolongation of shelf life than α-tocopherol and thus the order of antioxidant activity was once again proven: $\gamma > \delta > \alpha$. Complimentary to this, Satue *et al.* (1995) showed that α-tocopherol was not as good an antioxidant for the prevention of hydroperoxide formation as the other phenolic compounds, and at high concentrations showed a pro-oxidant effect.

Emulsification

Emulsions are divided into either water-in-oil or oil-in-water emulsions. In a water-in-oil emulsion the water droplets are dispersed in a semi-solid fat phase containing fat crystals and liquid oil (Gunstone, 1996). The emulsifying agents are of the hydrophobic type and other preservatives are normally added in the industry when this margarine-type emulsion is prepared (McClements & Decker, 2001).

In oil-in-water emulsions or in foods where oil droplets are dispersed into an aqueous matrix, oxygen must gain access to the lipid by diffusion into the aqueous phase and passage through the oil-water interface (Nawar, 1996). The rate of lipid oxidation in emulsions may be limited by the speed that free radicals, hydroperoxides or lipids can diffuse from one region to another within a droplet (McClements & Decker, 2001). This will depend on the interplay between a number of factors, including type and concentration of emulsifier, size of oil droplets, surface area of interface, viscosity of the aqueous phase, composition and porosity of the aqueous matrix, and pH (Nawar, 1996).

The most likely mechanism for the acceleration of lipid oxidation in emulsions is the decomposition of lipid hydroperoxides (ROOH) into the highly reactive peroxyl ($ROO^{\bullet}$) and alkoxyl ($RO^{\bullet}$) radicals by transition metals (reaction equation 6 & 7) or other pro-oxidants (McClements & Decker, 2001).

$$Fe^{3+} + ROOH \rightarrow Fe^{2+} + ROO^{\bullet} + H^{+} \quad \text{...6}$$

$$Fe^{2+} + ROOH \rightarrow Fe^{3+} + RO^{\bullet} + OH^{-} \quad \text{...7}$$

$$ROO^{\bullet} + LH \rightarrow ROOH + L \quad \text{...8}$$

$$RO^{\bullet} + LH \rightarrow ROH + L \quad \text{...9}$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad \text{...10}$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad \text{...11}$$

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{nonradical products} \quad \textbf{...12}$$

When oxidation occurs in an emulsion, these radicals react with unsaturated lipids (LH) within the droplets or at the oil-water interface, leading to the formation of lipid radicals ($L^{\bullet}$ and $LOO^{\bullet}$) (reaction equations 8 to 10). The lipid oxidation chain reaction propagates as these lipids react with other lipids in their immediate vicinity (reaction equation 11). Some of the lipid radicals formed may be terminated when they react with other radicals (reaction equation 12).

Light

The presence of any light, ultraviolet or γ-radiation is unfavourable and exposure to direct sunlight serves to enhance the effect of oxidation (Kritisakis, 1984). Fatty acids are colourless and photosensitised oxidation of unsaturated fatty acids is normally due to light absorption by colouring matter in the foodstuff when exposed to ultraviolet irradiation (Maloba *et al.*, 1996). Photo-oxidation involves the interaction between a double bond and highly reactive singlet oxygen produced from ordinary triplet oxygen by light in the presence of sensitisers such as chlorophyll, erythrosine, rosed Bengal or methylene blue.

When the oxidative stability of packaged vegetable oils was determined, coloured glass bottles had the best oxidative stability, followed by the clear glass and PET (Tawfik & Huyghebaert, 1997). The influence of light on oxidation was also evident, as the oxidative deterioration for olive oil covered in aluminium was slower than uncovered samples stored in clear glass bottles (Kritisakis, 1984).

D5 Determination of oxidative stability

It is obvious from the preceding discussion that lipid oxidation is an exceedingly complex process involving numerous reactions that cause a variety of chemical and physical changes. The nature and extent of each change is influenced by many variables that have been mentioned previously. Since oxidative decomposition is of major significance with regard to both the acceptability and nutritional quality of food products, many methods have been devised for assessing this occurrence (Nawar, 1996).

While there are numerous tests to measure the total fats and oils in foods, major controversy exists regarding the accuracy of test procedures and even how the total fat content should be defined (Marsili, 1993). Deciding which test results are most critical depends on the specific oils considered and how they will be used. Tests that monitor the extent of oxidation that has occurred in oil are among the most important and most common quality control tests (Hahm & Min, 1995). A single test, however, cannot possibly measure all oxidative events at once, nor can it be equally useful at all stages of the oxidative process, for all fats, all foods and all conditions of processing. At best, a test can monitor only a few changes as they apply to specific systems under specific conditions and for many purposes, a combination of tests is needed (Nawar, 1996).

A distinction should be made between susceptibility tests and methods of measuring the extent of lipid oxidation (e.g. peroxide value, conjugated dienoic value and *p*-anisidine value tests) (Marsili, 1993). Susceptibility tests measure the stability of a lipid under conditions that favour oxidative rancidity and include tests such as the Active Oxygen Method and Oxygen Stability Index. They are, therefore, based on tests that measure the extent of oxidation to provide an indication of the point at which oxidative rancidity occurs. Performing quality control tests to screen for potential oxidation problems, free fatty acid off-flavours, improper melting characteristics and undesirable mouth feel properties can go a long way in improving the quality of products (McClements, 2000).

Peroxide value

As hydroperoxides are the primary products formed as autoxidation commences and serve as precursors to the subsequent formation of secondary oxidation products, their presence and rate of change are important indicators of oil quality and potential shelf life (Ma *et al.*, 1997). A test commonly associated with routine quality control of edible fats and oils is the determination of the peroxide value (PV) after deodorisation, which thermally degrades residual hydroperoxides and strips out low molecular-weight aldehydes and free fatty acids (Hahm & Min, 1995). The efficacy of antioxidants in bulk oils in storage is mostly measured by means of PV determinations in conjunction with the Active Oxygen Method as a parameter of the shelf life of edible oils (Ma *et al.*, 1997).

The peroxide value is among the most common quality control tests (Marsili, 1993). This method determines all substances, in terms of milliequivalents of peroxide per thousand grams of sample, which oxidise potassium iodide under the conditions of the test (McClements, 2000). The substances are generally assumed to be peroxides or other similar products of fat oxidation. The standard AOCS PV determination (Cd 8 53, 1985) is based on the stoichiometric release of molecular iodine by hydroperoxides when exposed to KI in an acidic environment (Ma *et al.*, 1997). This reaction converts the hydroperoxides to alcohols. Soluble starch acts as an indicator when it is complexed with the molecular iodine released. This iodine is quantitated by titration with sodium thiosulfate. The higher the peroxide value, the more oxidised the fat is and the greater the likelihood of off-flavours (Marsili, 1993).

Freshly refined material should have a peroxide value below 1 meq.kg^{-1}. A fat is perceived as rancid at a peroxide level of about 10 meq.kg^{-1} (Gunstone, 1996). Although the PV test is empirical, it is also relatively simple, sensitive, reliable and reproducible if carried out under standardised conditions (Ma *et al.*, 1997). It was also found that the percentage weight gain during oxidation parallels the formation of hydroperoxides as monitored by PV measurements during the initial stages of oxidation (Wanasundara & Shahidi, 1994). This relationship changes as the hydroperoxides are broken down to secondary products during the later stages of oxidation. Although the thiobarbituric acid test and titration methods using other types of reducing agents as titrants are sometimes used, titration of iodine with sodium thoisulfate titrant is the most common procedure (Marsili, 1993).

Percentage conjugated dienoic acid

One of the first steps in the oxidation of linoleic acid (18:2) or higher polyunsaturated fatty acids (PUFA) in oil is a shift in the position of the double bonds (White, 1995). The greater the amount of PUFA in the oil, the greater the potential rise in conjugated dienes. Conjugated diene values of up to 6% are expected for high PUFA oils (Gunstone, 1996).

The main reaction involved in the oxidative deterioration of lipids is autoxidation, which is the reaction with molecular oxygen via a self-catalytic mechanism (Nawar, 1996). This situation causes the mobility of all the bonding electrons in the whole group composed of three vicinal carbon (-CH-, methyne) atoms, which can lead to a shift of the double bonds within this three-carbon unit or

allylic group (Hoffmann, 1989). The initial attack on a 1,4-pentadienoic system leads to free-radical formation on the methylene group between the two double bonds, which are shifted to form a conjugation of the two double bonds (Figure 2.6). This can be readily detected in the UV region of the spectrum by an absorption maximum around 232 nm (Nawar, 1996).

The CD value is expressed as a percentage of conjugated dienoic acid in the oil and is an indication of initial or primary oxidation products (White, 1995). The magnitude of change in CD values cannot be related to the degree of oxidation, especially when comparisons between oils of different fatty acid profiles or plant species are made (White, 1995).

The CD method measures primary oxidation products occurring during the oxidation of fats and oils, and its value correlates fairly well with PV (White, 1995). The advantages of this method over the PV method are that it is faster, simpler, requires less chemical reagents, does not depend upon a chemical reaction of colour development, and can be conducted on small sample sizes (White, 1995).

The CD method depends mainly upon the fatty acid composition of the fat or oil and cannot be applied to all studies of oil stability (White, 1995). The CD values thus cannot easily be compared from one type of oil to another, especially when large differences in the original fatty acid compositions occur. The method is useful, however, in measuring changes in oils containing substantial amounts of linoleic acid or more highly unsaturated fatty acids, such as most liquid vegetable oils (White, 1995).

As the CD method measures primary breakdown products occurring in the early stages of oxidation, the accumulation of these products parallels the uptake of oxygen and the formation of peroxides in the early stages of oxidation (Gunstone, 1996). The CD values, however, plateau at a certain concentration at which level the breakdown of formed CD presumably equals the formation of new CD compounds (White, 1995). It is at this point in oxidation that no further changes can be measured with this method. Iodine value

Another quality control test that is commonly used is the determination of the iodine value (Marsili, 1993). This test measures the amount of unsaturated fatty acids in the oil or fat. The higher the number, the softer the fat/oil; the lower the number, the harder the fat/oil. This type of test is most often applied when the softness or hardness of a product is important, for example in margarines and salad dressings.

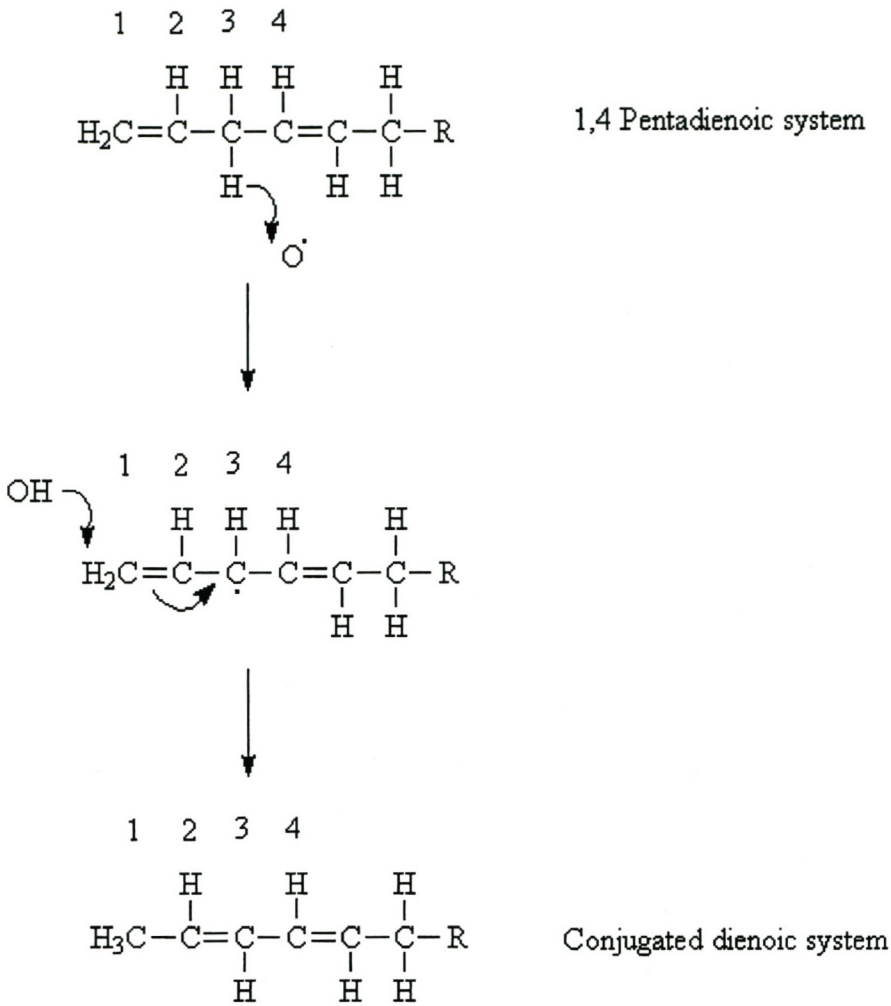


Figure 2.6. Formation of conjugated dienoic bonds by reaction with radical oxygen in a 1,4-pentadienoic system.

The iodine value is a good way to tell if the oil is unhydrogenated, partially hydrogenated or fully hydrogenated. By definition, the iodine value is the number of centigrams of iodine absorbed per gram of sample.

p-Anisidine value

Many of the compounds considered responsible for off-flavour development in oils are actually aldehydes, resulting in the development of the *p*-anisidine value (AV) to use the reaction of α and β aldehydes (mostly 2-alkenals) with *p*-anisidine reagent (Gunstone, 1996). The reaction, which takes places in acetic acid solution and the resulting Schiff base compounds, produces a yellowish colour that can be measured at a light absorbance of 350 nm (White, 1995).

Holm *et al.* (1957) initially developed the AV procedure, which was based on the reaction of aldehydes with benzidine acetate. Little is known about the relationship of the *p*-anisidine value with other methods of frying oil evaluation (Tompkins & Perkins, 1999). However, fried-food odour intensity, which is associated with t,t-2,4-decadienal, showed a highly significant correlation with the *p*-anisidine value. The *p*-anisidine value generally has a strong relationship with the techniques of headspace volatile analysis for all selected aldehydes, except nonanal ($r = 0.33$, $P=0.199$) and the sensory evaluation of overall odour intensity (Tompkins & Perkins, 1999). Nonanal is primarily formed by the breakdown of oleic acid, whilst the other aldehydes are primarily formed by the breakdown of linoleic and linolenic acids. A highly significant correlation is usually also observed between polymer content (polymerised acylglycerols) and *p*-anisidine values (Crapiste *et al.*, 1999). Tompkins & Perkins (1999) observed a correlation coefficient of 0.84 and a significance of 0.0001 between the polymer content and the *p*-anisidine value of soybean oil. Well-refined oils generally have *p*-anisidine values ranging between 1.0 and 10.0 $mmol.kg^{-1}$ fat (Crapiste *et al.*, 1999). Oils with high levels of polyunsaturated fatty acids (e.g. sunflower oil) can reach *p*-anisidine values of up to 46 $mmol.kg^{-1}$ when exposed to oxygen, while unexposed samples reach values up to 15 $mmol.kg^{-1}$ (Crapiste *et al.*, 1999).

The AV procedure was studied for its reproducibility and accuracy in analysing oils (White, 1995). Beers law held for a solution of 1-16 g of oil containing 0.1 – 10 µmol 2,4-dienals/100 ml oil (Holm, 1972). The AV procedure is simple, quick and can be performed directly on a solution of fat or oil (White, 1995).

Although anisidine values are not very sensitive and are not particularly suited for predicting off-flavours in oils, it can be used to monitor changes during oil processing, especially when the history of the oil is known. This test is probably most useful for the routine testing of crude oils to determine treatment before processing. The value obtained is also comparable only within each oil type, because initial AV varies among oil sources (White, 1995). Because the reaction does not require a high temperature or strong acid, the creation of artefacts from hydroperoxide breakdown is minimised (White, 1995).

Since peroxides are decomposed to give secondary products that are anisidine-reactive, it was possible to relate the AV to the PV (White, 1995). This was motivated by the fact that an increase by one PV unit corresponds to an increase of about two AV units (Holm *et al.*, 1957). This value was named the "TOTOX" value or the "Oxidation" value (OV), in which OV = AV + 2(PV) (Johansson & Persmark, 1971).

Because neither PV nor AV can independently adequately describe the extent of oxidative deterioration in an oil or fat, the combined effects of primary and secondary oxidative deterioration can be evaluated by calculating the total oxidation values (TOTOX) (Crapiste *et al.*, 1999).

Gas chromatography

The oxidative stability of fats and oils greatly depends on their fatty acid composition (Chu & Kung, 1998). Oils with high levels of unsaturation are more prone to oxidative deterioration due to structural changes caused by exposure to oxidative catalysing factors, especially light, heat and oxygen. During oxidation the physicochemical changes in oils are mostly related to the polyunsaturated fatty acids (Nawar, 1996). Linoleic and palmitic acids are usually used as indicators of the extent of fat deterioration because linoleic acid is more susceptible to oxidation, whereas palmitic acid is more stable towards oxidation (Tan *et al.*, 2001). During heating or exposure to oxygen, a decrease in the relative percentage of unsaturated fatty acids can be observed as oxidative deterioration commences.

Fatty acid composition can be examined by gas chromatography (GC), high-performance liquid chromatography (HPLC), and the use of silver ion systems (Gunstone, 1996). Fatty acids are too polar to chromatograph directly (Marsili, 1993) and are usually converted to methyl esters before injection into a gas chromatograph.

The method involves saponification of the fat to liberate the fatty acids from the triacylglycerides, followed by derivatisation of the fatty acid salts to form methyl esters (Gunstone, 1996). By analysing these methyl esters and monitoring the changes in fatty acid profile over a period of time, the oxidative state of fats and oils can be determined. Apart from the fatty acid profile, headspace volatile analysis of oxidised fats is also done to determine the level of rancidity and odour production correlated with oxidised oils (Gunstone, 1996).

The gas chromatography identification method for the analysis of fats and oils has also found application in forensic investigations concerning the adulteration of vegetable oils. Vegetable oils do not contain a wide fatty acid spectrum and these oils are easily falsified (Rezanka & Rezankova, 1999). Modern analytical methods, including capillary column chromatography, are used to disclose the adulteration of vegetable oils.

Near infrared spectroscopy

Near infrared spectroscopy (NIR) is based on the relatively weak and broad overtone and combination bands of fundamental vibrational transitions associated mainly with C-H, N-H and O-H functional groups (Shenk *et al.*, 1992).

The near infrared technique provides fast and accurate measurements of chemical components by measuring small samples in a non-destructive way (Osborne *et al.*, 1993) and giving information about structural and physical properties of the sample matrix. In addition, the NIR bands are less intense than mid infrared bands, which enables direct analysis of samples that are highly absorbent and scatters light strongly without dilution or extensive sample preparation (Shenk *et al.*, 1992; Hall *et al.*, 1996). Furthermore, the use of the absorption information obtained with an interferometer by Fourier Transform near-infrared spectroscopy improves spectral reproducibility and wave-number precision in comparison to results from dispersion instruments (McClure *et al.*, 1996).

The calibration of the spectrophotometer involves the activity of finding relationships between response variables (Y) and predictor variables (x) (Geladi, 2001). Although the multivariate calibration method is more complicated than the univariate calibration method, it is recommended as the more useful option. The main limitations of NIR spectroscopy are the strong dependence of reflectance on the

scattering properties of the sample and the existence of extensively overlapping absorption bands, which may confound any peak of interest (Osborne *et al.*, 1993).

The multivariate calibration method provides analyses with a means of overcoming problems by developing empirical models that relate the multiple spectral intensities from many calibration samples to the known analyte concentrations in these samples (Thomas & Haaland, 1990). Principal component regression (PCR) and partial least-squares regression (PLSR) are factor analysis methods that reduce the data at many frequencies to a relatively small number of intensities in a transformed full-spectrum coordinate system (Haaland & Thomas, 1988). PCR and PLSR have the potential to estimate the component concentration and chemical and physical properties from the NIR spectra.

Advances in Fourier transform near infrared spectroscopic instrumentation and multivariate data analysis techniques have significant potential for the determination of changes in food composition that may be indicative of the addition of extraneous material that could cause potential threats (Rodriguez-Saona *et al.*, 2000).

Work on the development of Fourier transform infrared (FT-IR) spectroscopy-based methods that allow analyses to be carried out directly on neat fat and oils and confer the advantages of analytical speed and automation has been done by the McGill IR Group (Ma *et al.*, 1997). The methods reported, include the FT-IR determination of peroxide value, measuring the characteristic O-H stretching absorption band of hydroperoxides. Fourier transform infrared spectroscopy (FT-IRS) has also been used in the determination of the PV and *cis* and *trans* fatty acid contents of fats and oils (Van de Voort *et al.*, 1994). To account for potential spectral interferences from other OH-containing species that may be present in oils, such as alcohols, moisture, free fatty acids and mono- and diglycerides, a PLSR multivariate calibration approach was used by Ma *et al.* (1997). The main limitation of this method was its lack of sensitivity, with peroxide values of less than 1.5 effectively being undetectable. The complexity, as well as the lack of sensitivity was considered a hurdle to the implementation of this method (Ma *et al.*, 1997).

Yildiz *et al.* (2001) have, however, reported successful calibration models for the prediction of peroxide values, conjugated diene values and *p*-anisidine values of oxidised oils. The best calibrations for all three methods were obtained using PLS regression and first derivative, yielding correlations of 0.989, 0.916 and 0.938

respectively. The standard error of prediction (SEP) for models predicting the PV, CD and AV was 0.938 meq.kg^{-1}, 0.025 % and 0.95 mmol.kg^{-1} respectively.

Near infrared (NIR) spectroscopy has been used as an authenticity-testing tool as a means to differentiate among vegetable oil types (Bewig *et al.*, 1994). It has also been applied in the determination of free fatty acids (FFA), iodine value, and the fatty acid composition of fats and oils (Sato *et al.*, 1991, Che Man & Moh, 1998).

Conclusion

The oxidative changes occurring in edible fats and oils during storage have successfully been examined using the peroxide value, conjugated diene value and *p*-anisidine value tests. Values obtained from these tests have, in turn, been used as reference values to build calibration models for predicting the oxidative stability of edible oils using Fourier transform near infrared spectroscopy. The monitoring of changes in the fatty acid profile of these edible fats and oils over a period of time have been determined with methods including gas chromatography, high pressure liquid chromatography and thin layer chromatography. Oxidative decay of unsaturated fatty acids has been accepted as an indicator of oxidative status of fats and oils. The prediction of oxidative decay with FT-NIRS using gas chromatography as a reference method to monitor the changes in fatty acid profile have, however, not been implemented.

The oxidative changes occurring in mango kernel fat (MKF) during storage under different conditions have not yet been studied or defined. Although the fatty acid profile of MKF has been studied, none of the fat obtained from the kernel of *Mangifera indica* grown in South Africa has been documented. Consequently, the changes in fatty acid profile occurring during storage have not been studied. No attempts have been made to predict the oxidative stability of MKF with FT-NIRS or any other form of spectroscopic calibration and prediction methods.

The peroxide value, conjugated diene value and *p*-anisine value tests are all very reliable and repeatable methods for determining the oxidative stability of edible fats and oils over a period of time and can be successfully implemented as reference methods when building calibration models using FT-NIRS. The successful implementation of FT-NIRS for predicting the oxidative stability of edible fats and oils is the more cost and time-effective alternative to conventional time consuming methods.

The oxidative stability of mango kernel fat needs to be examined before the fat can be marketed as either a raw or secondary product. This will aid in the utilisation of this source of edible fat, which is traditionally considered to be a waste product.

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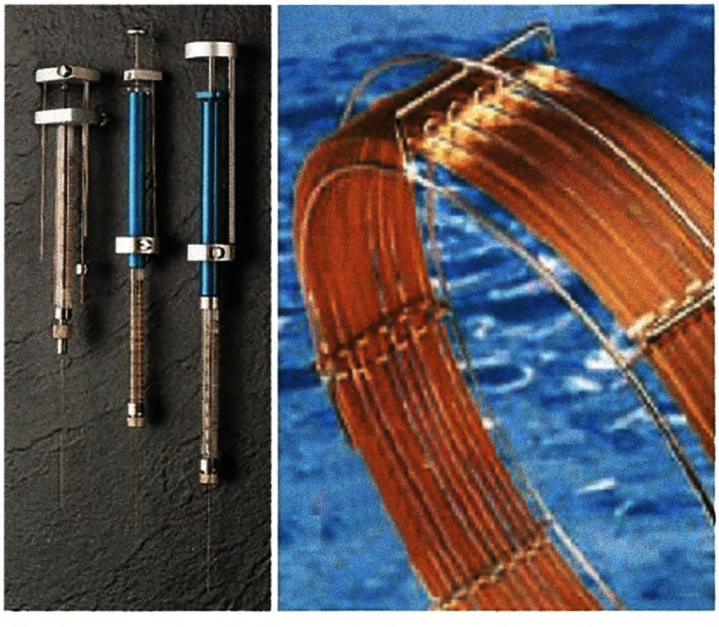
CHAPTER 3

MONITORING THE CHANGES IN FATTY ACID PROFILE OF MANGO (*Mangifera indica* L.) KERNEL FAT DURING STORAGE UNDER DIFFERENT CONDITIONS BY MEANS OF GAS CHROMATOGRAPHY



Chapter 3

MONITORING THE CHANGES IN FATTY ACID PROFILE OF MANGO (*Mangifera indica* L.) KERNEL FAT DURING STORAGE UNDER DIFFERENT CONDITIONS BY MEANS OF GAS CHROMATOGRAPHY

Summary

The fatty acid methyl esters identified in mango kernel fat (MKF) by gas chromatography were, palmitic (C16:0; 8.34%), stearic (C18:0; 34.98%), oleic (C18:1; 48.05%), linoleic (C18:2; 6.60%), linolenic (C18:3; 0.43%) and arachidic (C20:0; 1.73%). Trace amounts of C16:1, and C22:0 were also found. The mango kernel fat samples were stored exposed and unexposed to a limited amount of oxygen, at 5, 15, 25 and 40°C. Changes in the fatty acid profile of the stored samples were monitored over a period of 240 days at 40-day intervals. The percentage of linoleic acid decreased significantly from 6.60% to 5.28% after 40 days, but thereafter remained relatively stable with slight decreases up to 240 days. The trace amounts of C18:3 also decreased during the storage period of 240 days. The saturated fatty acids (C16:0 & C18:0), as well as the oleic acid (C18:1) showed stability over the period of 240 days. The values obtained from samples stored at different temperatures showed little variation. The minimal influence of the limited amount of oxygen on the oxidative deterioration of the mango kernel fat was also evident. The high α-tocopherol content of MKF (100mg/100g^{-1} fat) might have had an influence on the oxidative stability of the fat.

Introduction

The oxidative deterioration of fats and oils is dependant mainly on their chemical composition, the concentration of minor compounds with antioxidant or pro-oxidant characteristics, the degree of processing and storage conditions, especially relating to temperature and oxygen concentration (Koskas *et al.*, 1984). In edible oils, the susceptibility to oxidation increases geometrically in relation to the degree of unsaturation and ultimately, it is the chemical structure of a lipid molecule that determines its susceptibility to oxidation, particularly the number and location of the double bonds (Ulberth, 1997).

The important lipids in oxidation reactions are those containing unsaturated fatty acids, particularly oleic (C18:1), linolenic (C18:3) and linoleic (C18:2) acid, which is the most susceptible to oxidation (Frankel & Huang, 1994; White, 1995). The order of decreasing positive influence of fatty acid composition on oxidative stability is palmitic acid, followed by stearic acid and oleic acid (Chu & Kung, 1998). The relative rate of the autoxidation of fatty acids is approximately 100:1 for oleic:stearic acids and 1200:1 for linoleic:stearic acids, at 20°C (Evans, 1997).

Hydroperoxides, generally referred to as peroxides, are the major initial reaction products of unsaturated fatty acids with oxygen (McClements, 2000). These intermediate compounds are highly reactive in the development of off-flavour lipid oxidation aromas (Marsili, 1993). The flavour compounds produced, appear to depend on the type of oxidation mechanism, the hydroperoxides formed, and the nature of the fatty acids involved. Palm oil, for example, is not very susceptible to oxidation, because it is high in oleic acid (*ca.* 35%), low in linoleic acid (*ca.* 10%) and linolenic acid is almost negligible (*ca.* 0.2%) (Clegg, 1973).

The chemical analyses that determine the oxidative status of oils effectively include peroxide value, anisidine value, free fatty acid content, iodine value and C18:2/C16:0 peak area ratio. Peak area ratios can be determined by gas chromatography (GC), high performance liquid chromatography (HPLC), thin layer chromatography (TLC) gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC) and liquid chromatography-Fourier transform infrared spectroscopy (LC-FTIR) (Gunstone, 1996).

As oxidation advances, a continuous decrease in unsaturated fatty acids, particularly linoleic acid, can usually be observed (Crapiste *et al.*, 1999). Linoleic and palmitic acids are generally used as indicators of the extent of fat deterioration, because palmitic acid is more stable to oxidation than linoleic acid (Tan *et al.*, 2001). Tan *et al.* (2001) also reported a decrease in the relative percentage of unsaturated fatty aids (C18:2) and an increase in the relative percentage of saturated fatty acids (C16:0) after microwave heating of corn oil and soybean oil. Purdy (1985), however, showed that the stability of high oleic-containing sunflower and safflower oils increased directly in relation to their C18:1 content.

In addition to the fatty acid composition, the other component responsible for oxidative stability in edible oils is the tocopherol content (Chu & Kung, 1998). High percentages of α-tocopherol have shown pro oxidant activity (>500 ppm = 50

necessary to allow filling of the vials before re-solidification of the fat, which normally starts at 27°C, occurred (Figure 3.1). Headspace was allowed in 50% of the samples by filling the vials with approximately 16 ml of the fat and sealing them with aluminium crimp caps and Teflon seals. The rest of the samples were filled to the top to ensure total oxygen exclusion. Samples were stored in the dark at 5, 15, 25 and 40°C respectively for 240 days. No vial, once removed from storage and used for analysis, was reused.

At 40-day intervals, samples from all three batches, stored at the respective temperatures, were liquefied at a temperature of *ca.* 45°C for 10 minutes in preparation for analysis. This led to an easily obtainable, more manageable homogenous oil sample. These samples were subsequently used to create methyl esters for the GC analysis. Analyses were performed on all samples, stored with and without oxygen, at 5, 15, 25 and 40°C respectively for 240 days.

Sample preparation for gas chromatographic analysis

Screw cap glass test tubes were washed with Extran phospolipid-free soap and rinsed with distilled water to ensure the removal of all possible traces of metals and lipids. The C17:0 internal standard (T-2151 Triheptadecanoin, SIGMA) was weighed (6.25 mg) into each of the 10 ml screw cap test tubes. Mango kernel fat (0.05 g) and 5 ml of a 10% KOH in CH_3OH solution were added to each test tube. The tubes were purged with nitrogen gas, sealed tightly and placed in a water bath at 85°C. After two hours, the test tubes were removed from the water bath, again purged with nitrogen and stored overnight at –18°C. After 5 minutes at room temperature, 1 ml of concentrated HCl was added to each test tube. Extractions were made with 5 ml of hexane and BHT (5 ml of 0.05 g butylated hydroxy toluene (BHT) diluted in 500 ml hexane). To complete the first extraction, the samples were centrifuged at 4000 rpm (Beckman model TJ-6 centrifuge) and the hexane phase was pipetted into a clean test tube. A second extraction was carried out with 5 ml of pure hexane. The 10 ml of hexane in each test tube was allowed to evaporate in a 35°C water bath using nitrogen gas. After evaporation, 2 ml of a 14% H_2SO_4 in CH_3OH solution was added to each test tube, the tubes were purged with nitrogen gas and then placed in a water bath for 2 hours at 80°C. The tubes were removed from the water bath and allowed to cool

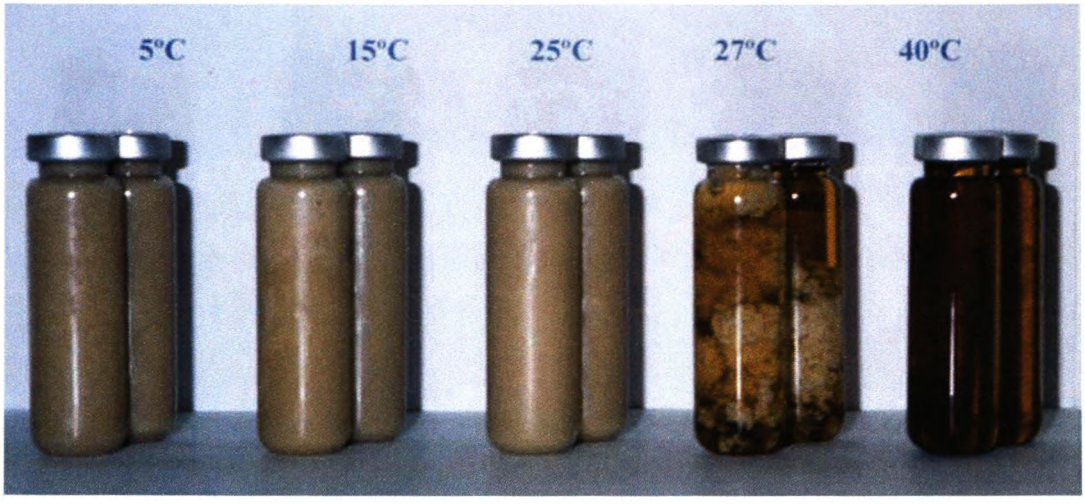


Figure 3.1. The physical characteristics of mango kernel fat at different temperatures.

down before 2 ml of water was added. Consecutive hexane extractions were repeated as before. The test tubes, filled with 10 ml hexane containing the methyl esters were stored at –80°C until injected. Just before injection into the GC, the hexane was allowed to evaporate from each test tube. A dilution of 1 μl oil in 1 ml of CS_2 was made in a 1.5 ml GC bottle, of which 0.3 μl was injected into the gas chromatograph.

Gas chromatographic analysis of long chain fatty acid methyl esters

Long chain fatty acid methyl esters were determined using a Fisons series 8000 GOS chromatograph equipped with a flame ionisation detector and a bonded phase DB-23 fused silica capillary column (J&W Scientific, USA) of 30 m x 0.25 mm. The initial column temperature of 50°C was increased at a rate of 10°C per minute to 150°C, then increased at a rate of 4°C per minute to 240°C and held for 10 minutes. The injector temperature was set at 240°C, while the detector temperature was set at 270°C. Helium was used as carrier gas with a linear flow rate of 1.7 ml.min.$^{-1}$ (28.5 cm^{-1}). A split ratio of 1:5 was used. The fatty acid profile was calibrated with the 18920-1AMP fatty acid methyl ester mix external standard (SUPELCO chromatography products).

Gas chromatography-mass spectroscopy

A Carlo Erba QMD 1000 quadropole GC-MS system, connected to a PS 089 40 m x 0.25 mm capillary system with a 1.0 μm film thickness (equal to a HP5 or DB 5) produced by the laboratory for Ecological Chemistry, University of Stellenbosch, was used to confirm the identity of the fatty acids. The injector temperature was 220°C with an ion source temperature of 200°C and electron impact ionisation of 70eV. Helium was used as carrier gas with a linear flow rate of 28.5 cm.s^{-1} (*ca.* 1.7 ml.min^{-1}) and a split ratio of *ca.*1:10 was employed. The initial temperature of 40°C was increased to 280°C at a rate of 4°C.min^{-1}. Peaks in the mass spectra were identified by means of an NBS mass-spectral library.

Vitamin E content analysis

The vitamin E content was determined using the high performance liquid chromatography (HPLC) method for the routine determination of tocopherols in animal feed and human foodstuffs (Manz & Phillipp, 1981). Analysis was performed

by ARC – Animal Nutrition and Animal Products Institute, Private Bag X2, Irene, 0062.

Statistical analysis

Graphs were compiled using Statistica version 6. Every point on the graph indicates the average value calculated from two duplicates in three batches (six values). The bar indicated at each value represents the 95% confidence interval for the average. An inspection of the overlapping of the 95% confidence intervals identified significant changes. When no overlapping occurred, significant differences were assumed.

Results and discussion

The chromatogram displaying the fatty acid profile of the crude, cold-pressed mango kernel fat is depicted in Figure 3.2. The fatty acid methyl esters were identified by GC-MS analysis and the corresponding total ion chromatogram (TIC) is shown in Figure 3.3. The fatty acid profile of the mango kernel fat was determined as C16:0 (8.43%), C16:1 (0.56%), C18:0 (34.98%), C18:1 *trans* (0.25%), C18:1 *cis* (48.05%), C18:2 (6.60%), C18:3 (0.43%), C20:0 (1.73%), C20:1 (0.25%) and C22:0 (0.40%) (Figure 3.4). These results were similar to values obtained by Lakshiminarayana *et al.* (1983) and Van Pee *et al.* (1981) (Table 3.1).

The different storage temperatures (5, 15, 25 and 40°C) had a minimal effect on the concentration of the fatty acids, therefore the fatty acid concentrations at these temperatures were combined (Figures 3.5 & 3.6) to attain an average value for every fatty acid. This minimal effect could be due to the fact that lower storage temperatures were employed as studies of oxidative stability at high temperatures cannot be extrapolated to ambient temperatures (Satue *et al.*, 1995) and the MKF is seldom stored at temperatures higher than 40°C. Inclusion and exclusion of oxygen also demonstrated little variation, as can be seen from the results (Figures 3.7 & 3.8) and consequently these results were also combined to create a larger data set with more representative samples.

Since the polyunsaturated fatty acid content was low (*ca.* 7%) and the tocopherol content was measured at *ca.* 100 mg.100g^{-1} fat, the MKF was expected to

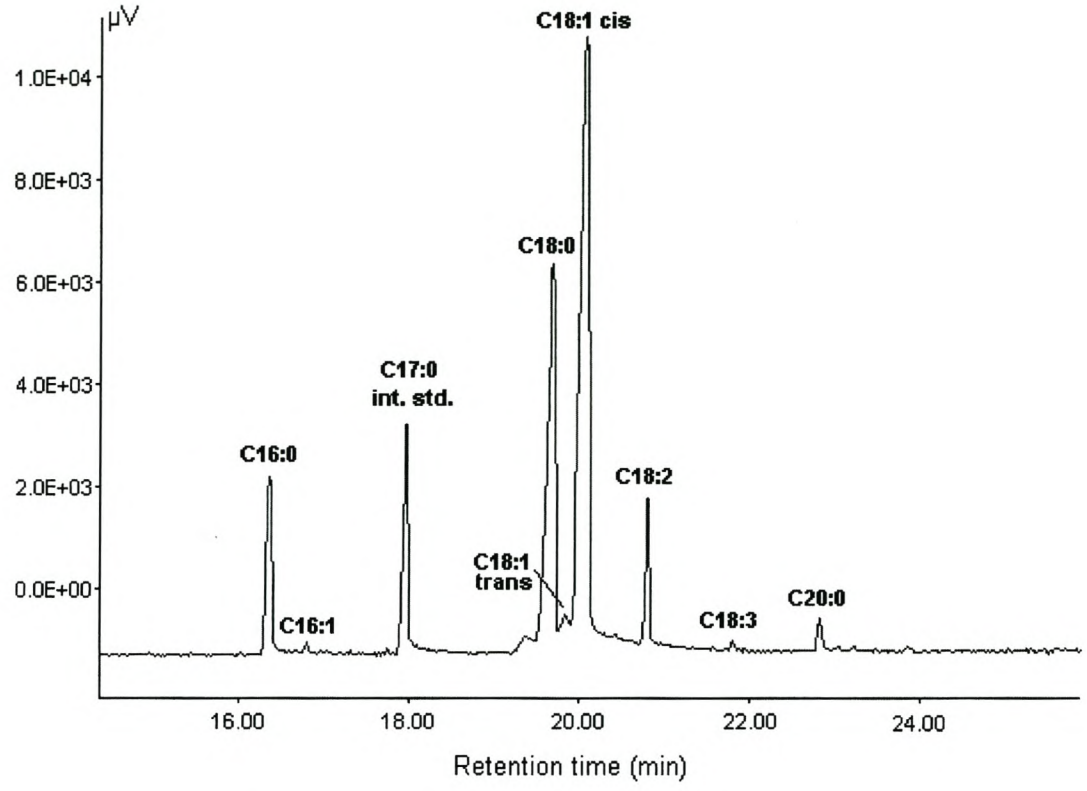


Figure 3.2. Gas chromatogram of fatty acid methyl esters derived from the fatty acids present in mango kernel fat.

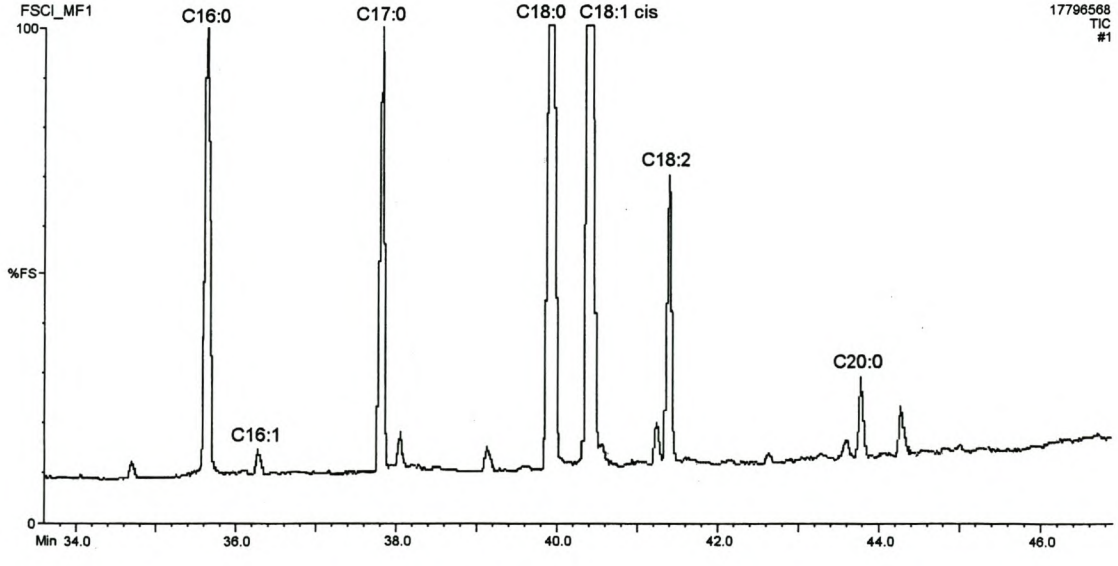


Figure 3.3. Total ion chromatogram of the fatty acid methyl esters of mango kernel fat as identified by GC-MS analysis.

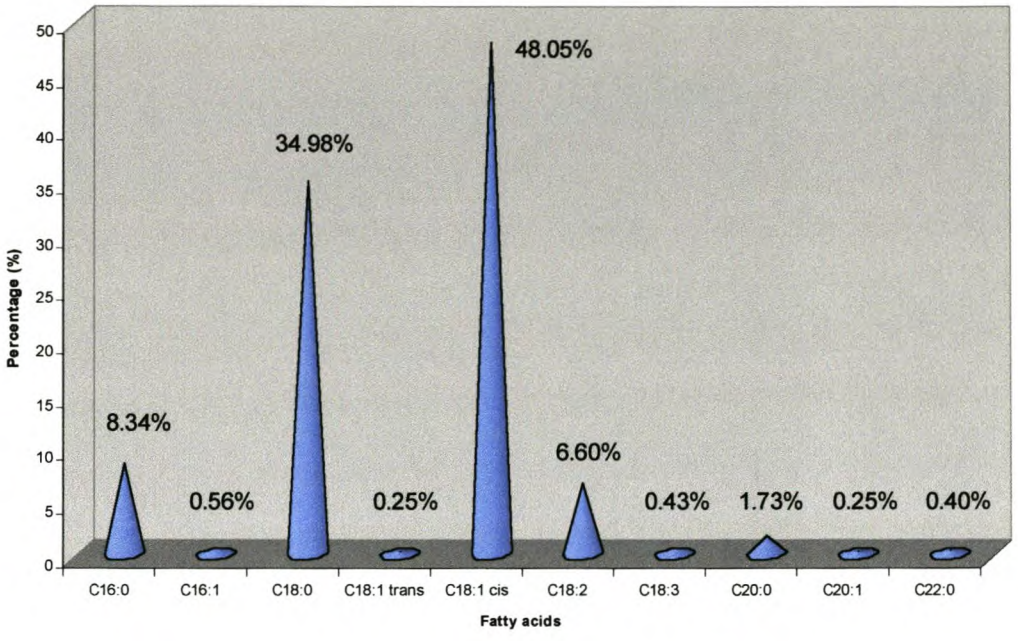


Figure 3.4. Graphic representation of the fatty acid distribution in crude, cold-pressed mango kernel fat (MKF); also showing trace fatty acids

Table 3.1. Fatty acid profile of crude, cold-pressed mango kernel fat as obtained in present and previous studies

		Present study	**Lakshiminarayana *et al.* (1983)**	**Van Pee *et al.* (1980)**
Fatty Acids		*Percentage of total fatty acids (%)*		
Palmitic	C16:0	8.34	3 - 18	5.9 – 9.1
Stearic	C18:0	34.98	24 – 57	32 – 44
Oleic (*trans*)	C18:1	0.25		
Oleic (*cis*)	C18:1	48.05	34 – 56	43.7 – 54.5
Linoleic	C18:2	6.60	1 – 13	3.6 – 6.7
Linolenic	C18:3	0.43		0.3 – 1.3
Arachidic	C20:0	1.73	1 - 4	0.5 – 3.6

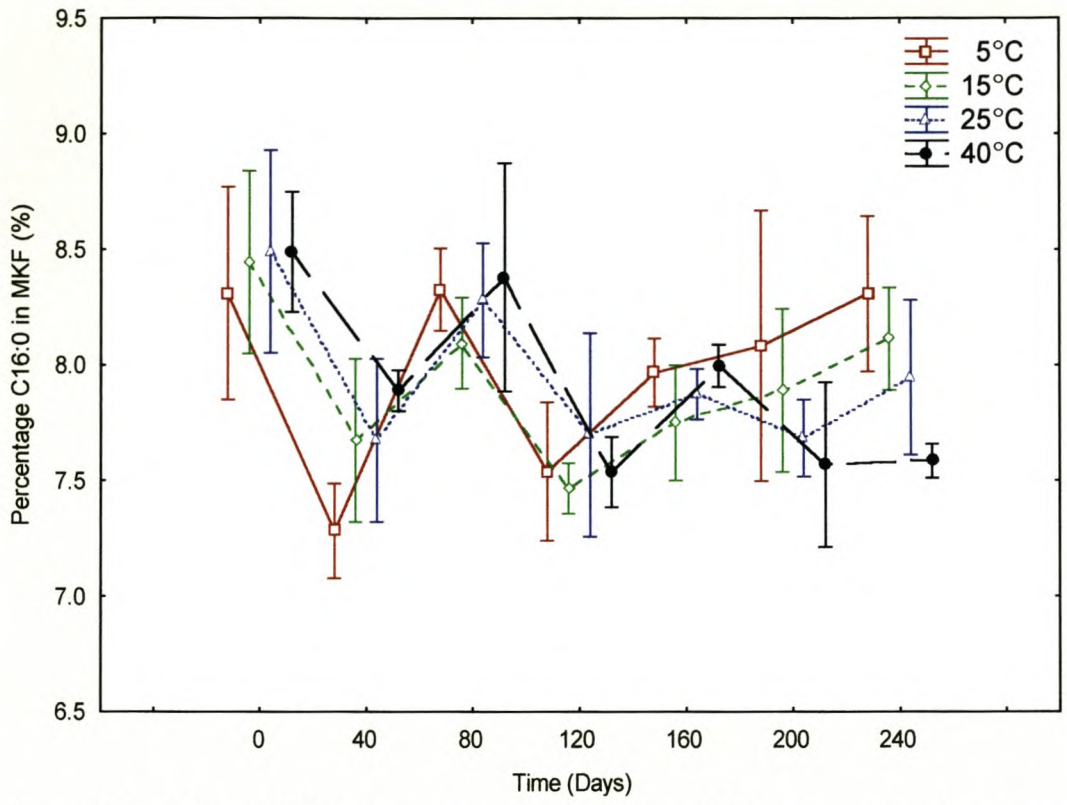


Figure 3.5. Effect of different storage temperatures with headspace on the percentage of palmitic acid (C16:0) in mango kernel fat (MKF) over 240 days.

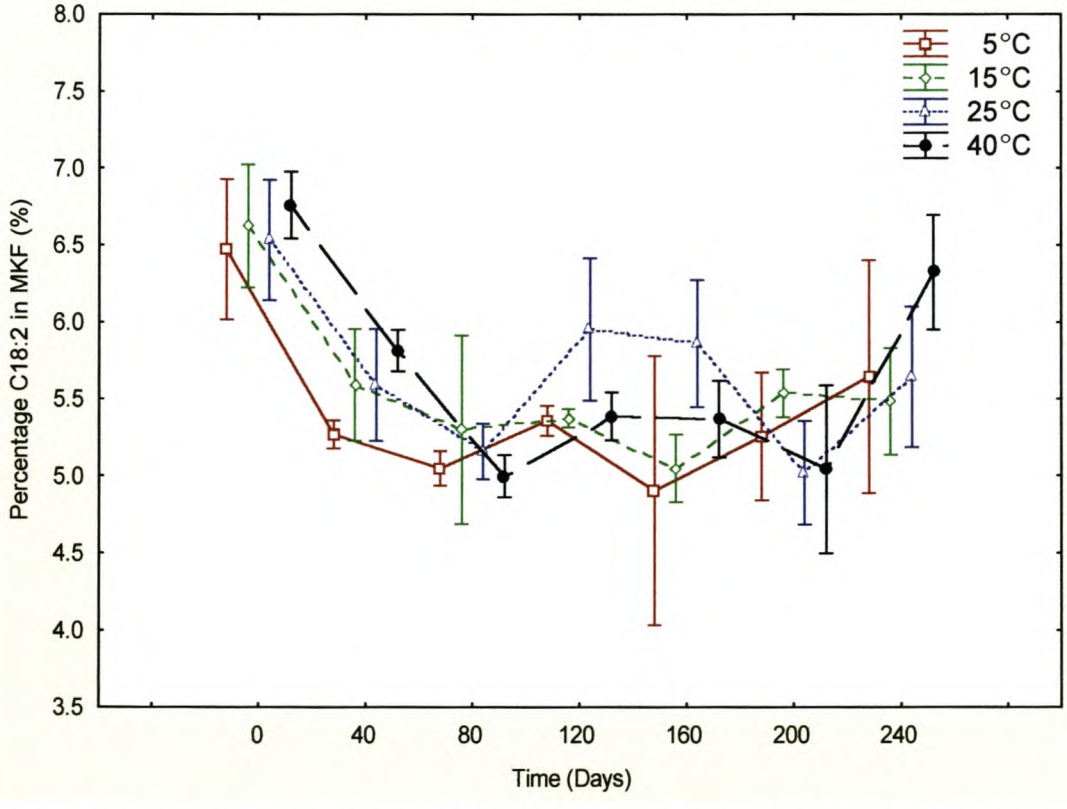


Figure 3.6. Effect of different storage temperatures with headspace on the percentage of linoleic acid (C18:2) in mango kernel fat (MKF) over 240 days.

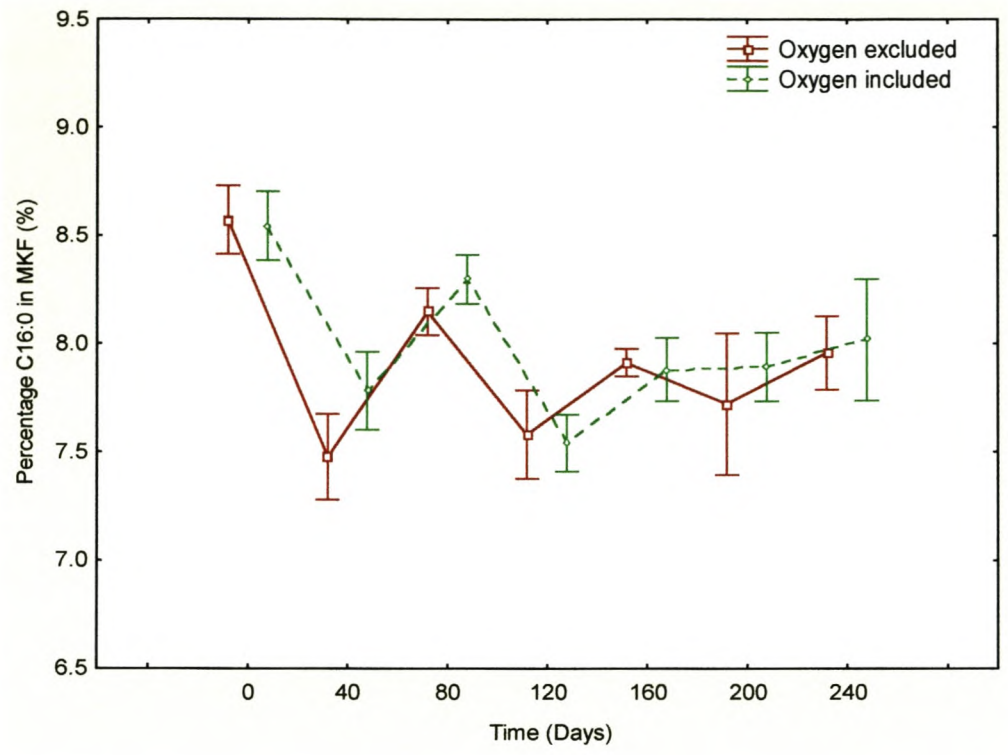


Figure 3.7. Effect of limited oxygen inclusion in the headspace on percentage of palmitic acid (C16:0) in mango kernel fat (MKF) during storage over 240 days.

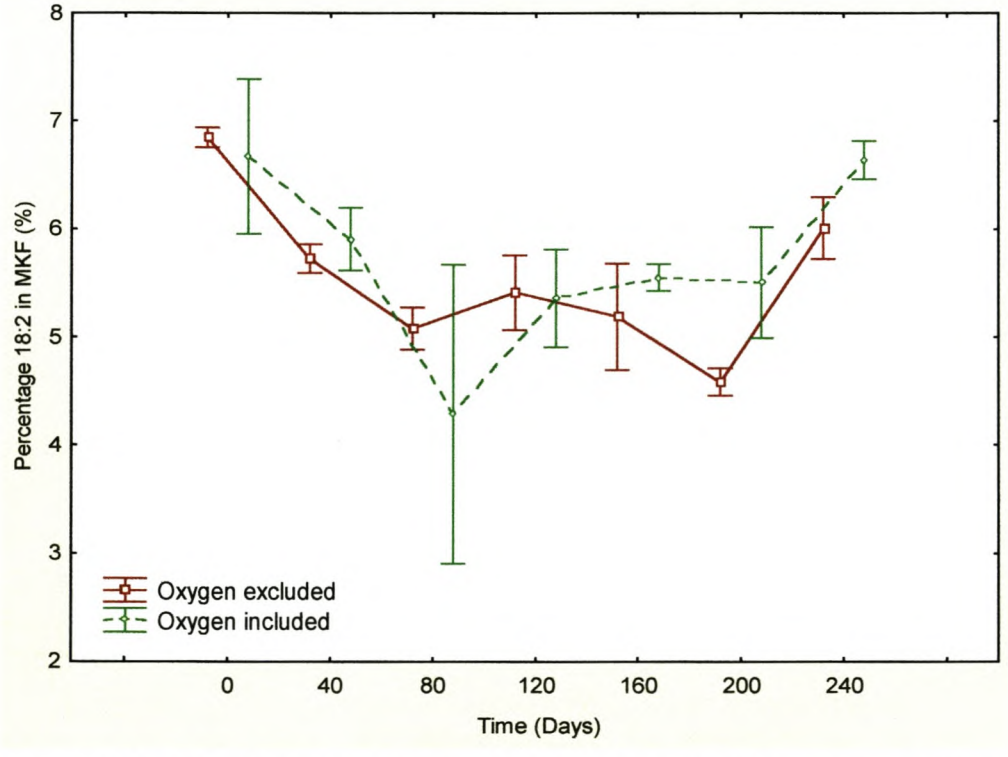


Figure 3.8. Effect of limited oxygen inclusion in the headspace on percentage of linoleic acid (C18:2) in mango kernel fat (MKF) during storage over 240 days.

remain stable against oxidative deterioration during the shelf life study. It was therefore expected that a decline in C18:3 and C18:2 would be observed, while saturated fatty acids (C16:0 & C18:0) and C18:1 were expected to remain stable between 0 and 240 days. The average decrease in C18:2 and C18:3 fatty acid content over a period of 240 days is depicted in Figures 3.9 and 3.10 respectively. Linoleic acid (Figure 3.9) decreased drastically at 40 days, whereafter the levels remained constant up to 240 days, with only small biological variation, which can be neglected due to the 95% confidence intervals. It seems as if primary oxidation was completed after 40 days, as no further degradation of the linoleic and linolenic acids was evident after this point.

The C18:3 content of MKF was measured as trace amounts, resulting in the expected uneven distribution (Figure 3.10). Despite this, a declining trend could be observed between days 0 and 240. As expected, the average values for C18:1(*cis*), determined over a period of 240 days demonstrated a very slight decrease (Figure 3.11). This fatty acid also behaved in a stable manner on comparison to linoleic acid. Although C16:0 (palmitic acid) was expected to remain stable, a decrease that can be attributed to biological variation was visible over a period of 240 days (Figure 3.12). This variation seemed to be pronounced throughout the analysis of the MKF and may be ascribed to the crude nature of the fat. Stearic acid (C18:0) seemed to increase slightly over the period of 240 days (Figure 3.13). The peak area ratios of C18:2/C16:0 and C18:2/C18:0 were calculated. The changes observed in the these fatty acid ratios confirmed that polyunsaturated fatty acids were more prone to oxidative deterioration than saturated fatty acids (Figures 3.14 & 3.15). Decreases of 7.41% and 12.80% were observed between day 0 and 240 for the C18:2/C16:0 and C18:2/C18:0 ratios, respectively.

The small differences in the results for samples stored with and without the inclusion of air indicate the need for further studies including larger headspace and different oxygen concentrations. The oxygen present in the samples did not have a pronounced effect on the oxidation of the fat and can be explained by equations 3.1 to 3.3.

Assume 16 ml of an oil containing the glyceride, trioleic acid is kept in an airtight container with 9 ml of headspace (air = 79% N_2 + 20% O_2). It is known that the density of triolein is 0.916 $g.ml^{-1}$ and that it has a molecular weight of 885.5 $g.mol^{-1}$ (Burger, (2002).

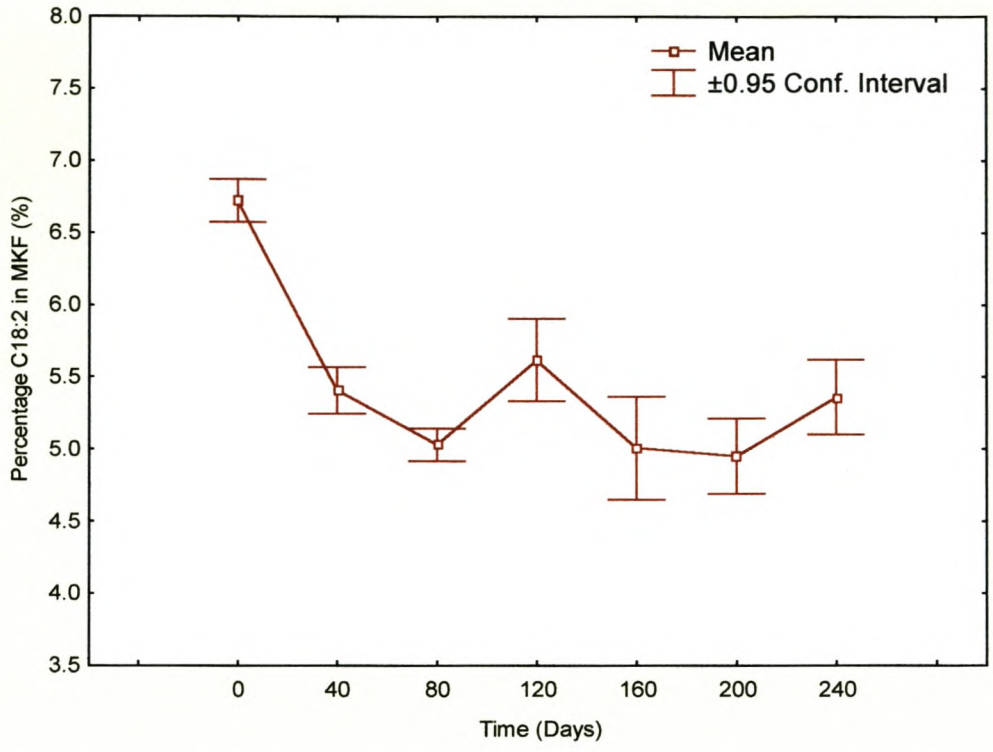


Figure 3.9. Effect of storage over 240 days on percentage of linoleic acid (C18:2) in mango kernel fat (MKF).

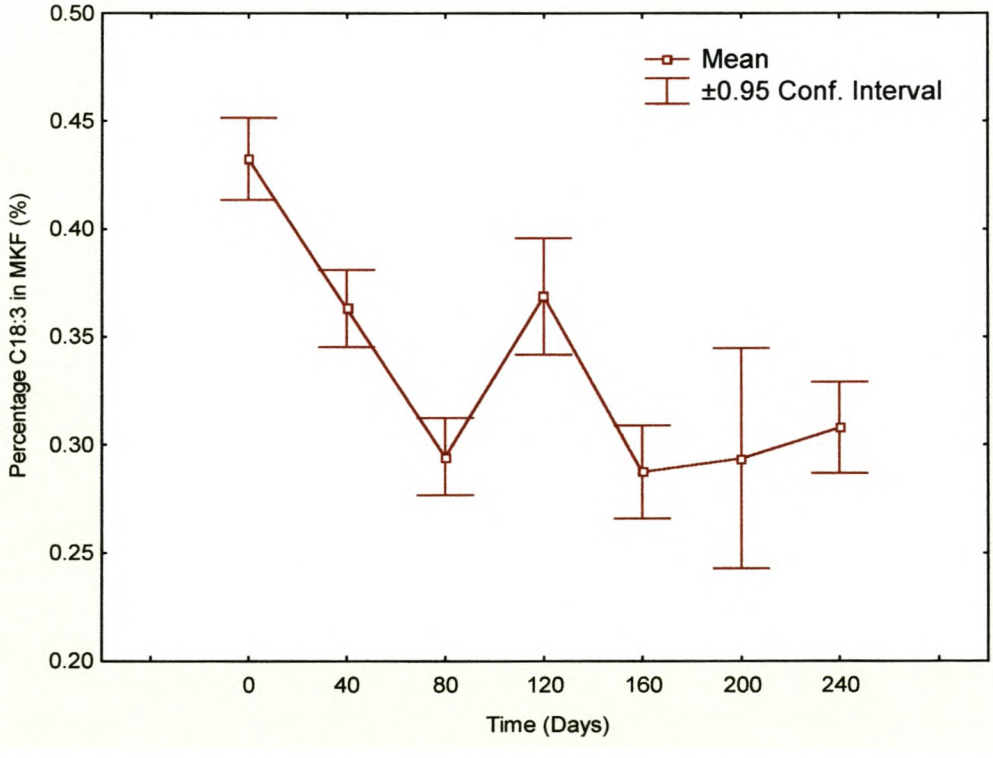


Figure 3.10. Effect of storage over 240 days on percentage of linolenic acid (C18:3) in mango kernel fat (MKF).

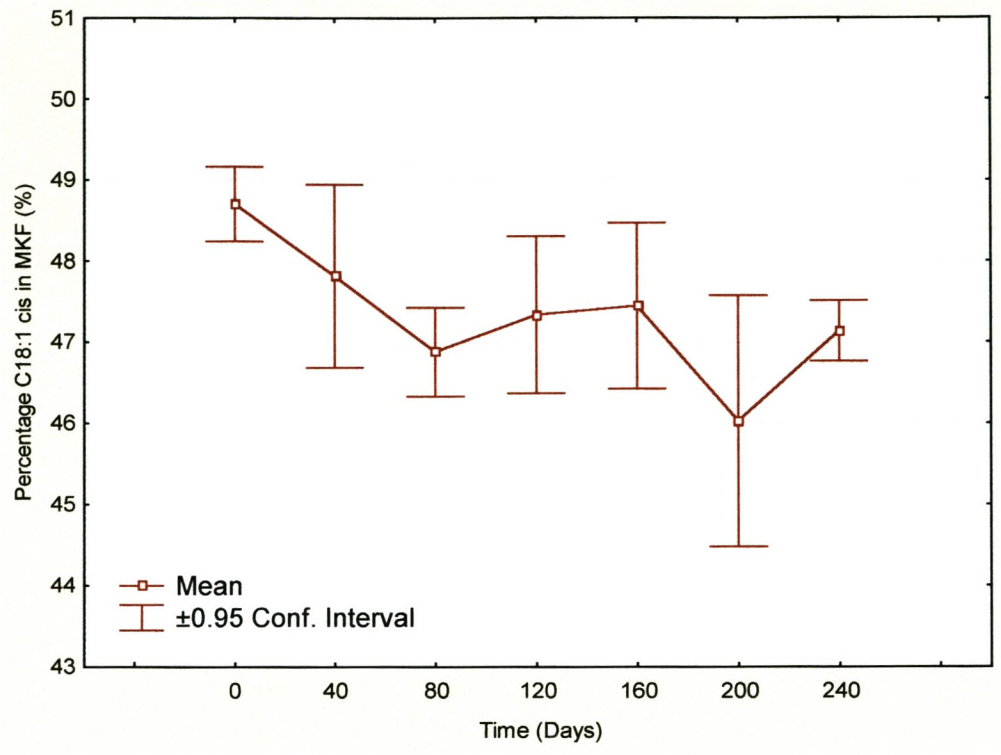


Figure 3.11. Effect of storage over 240 days on percentage of oleic acid (C18:1(*cis*) in mango kernel fat (MKF).

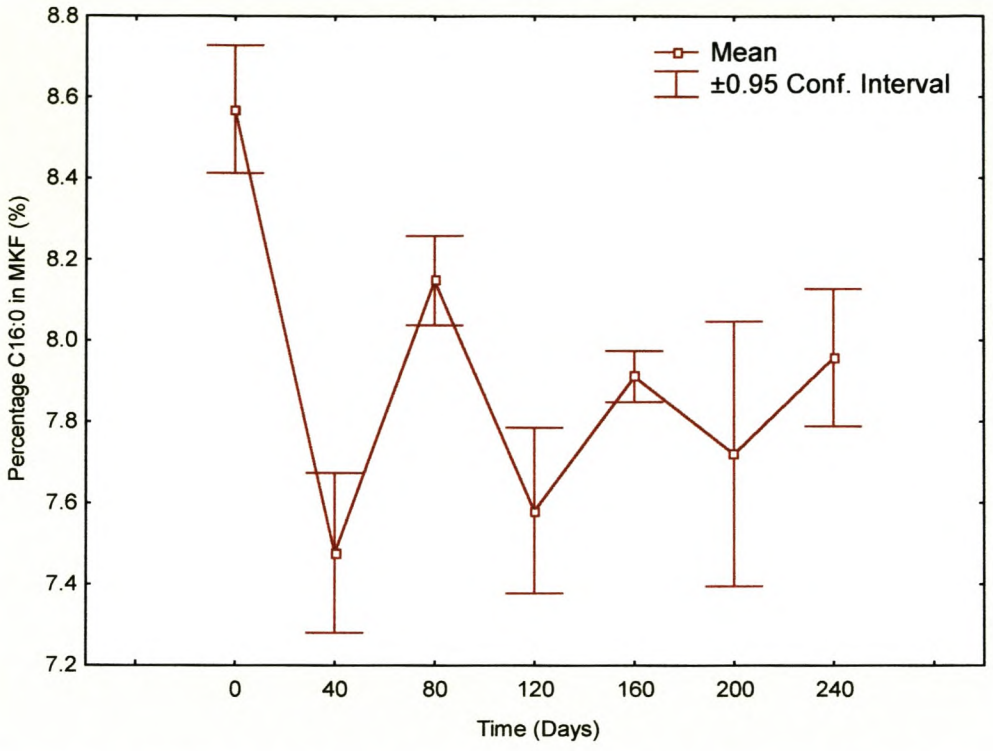


Figure 3.12 Effect of storage over 240 days on percentage of palmitic acid (C16:0) in mango kernel fat (MKF).

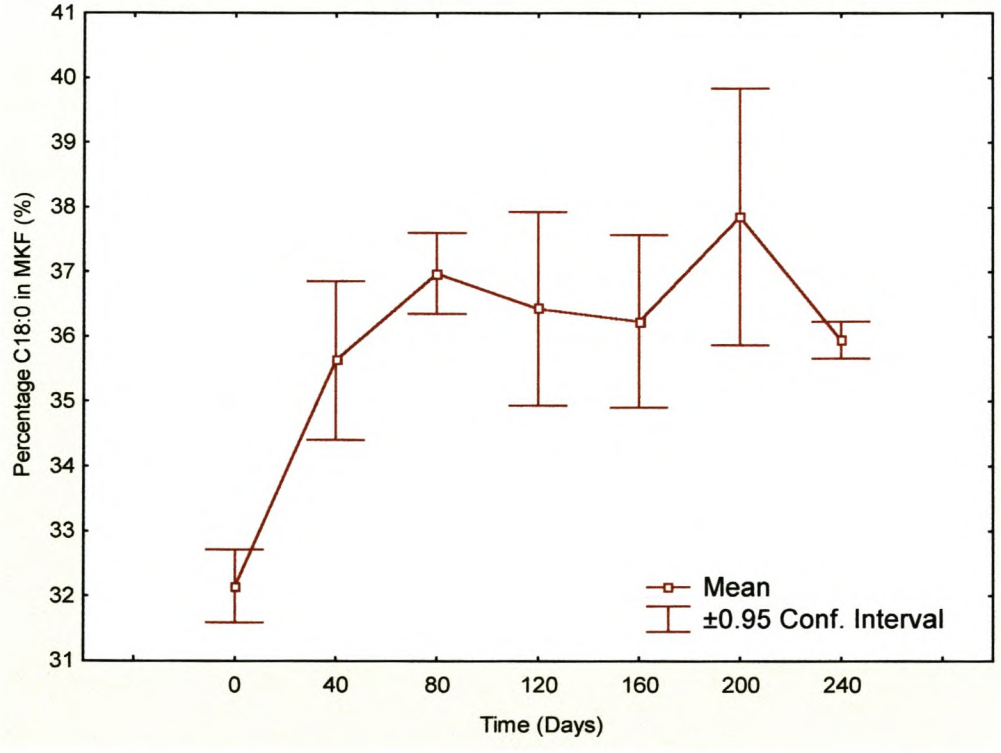


Figure 3.13. Effect of storage over 240 days on percentage of stearic acid (C18:0) in mango kernel fat (MKF).

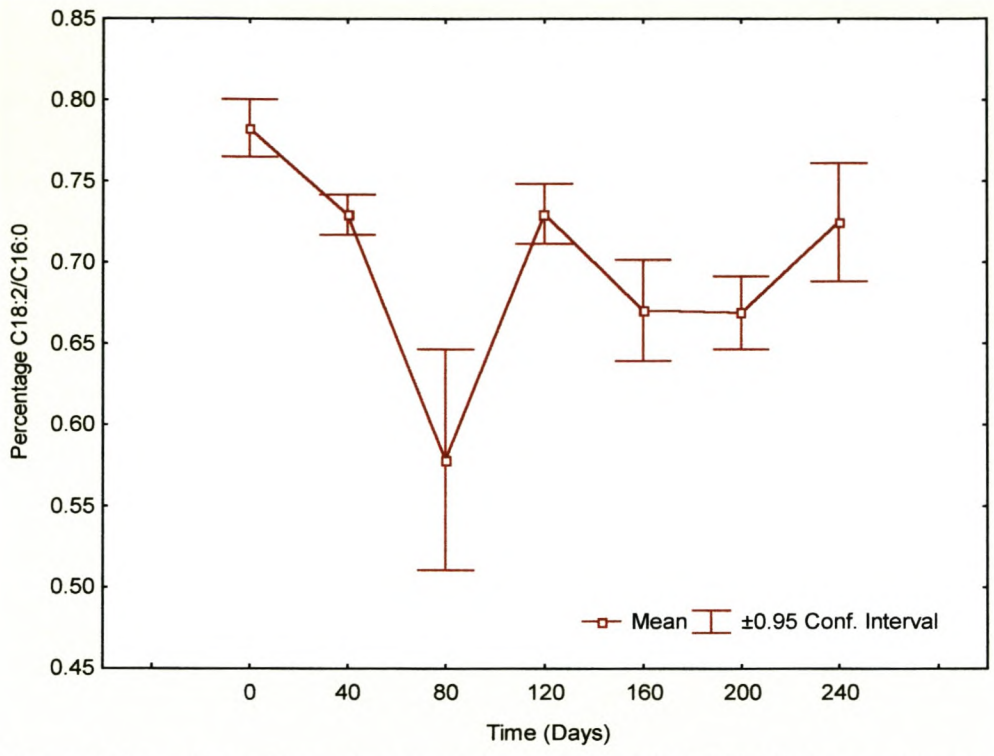


Figure 3.14. The effect of storage time on the C18:2/C16:0 fatty acid ratio of mango kernel fat (MKF).

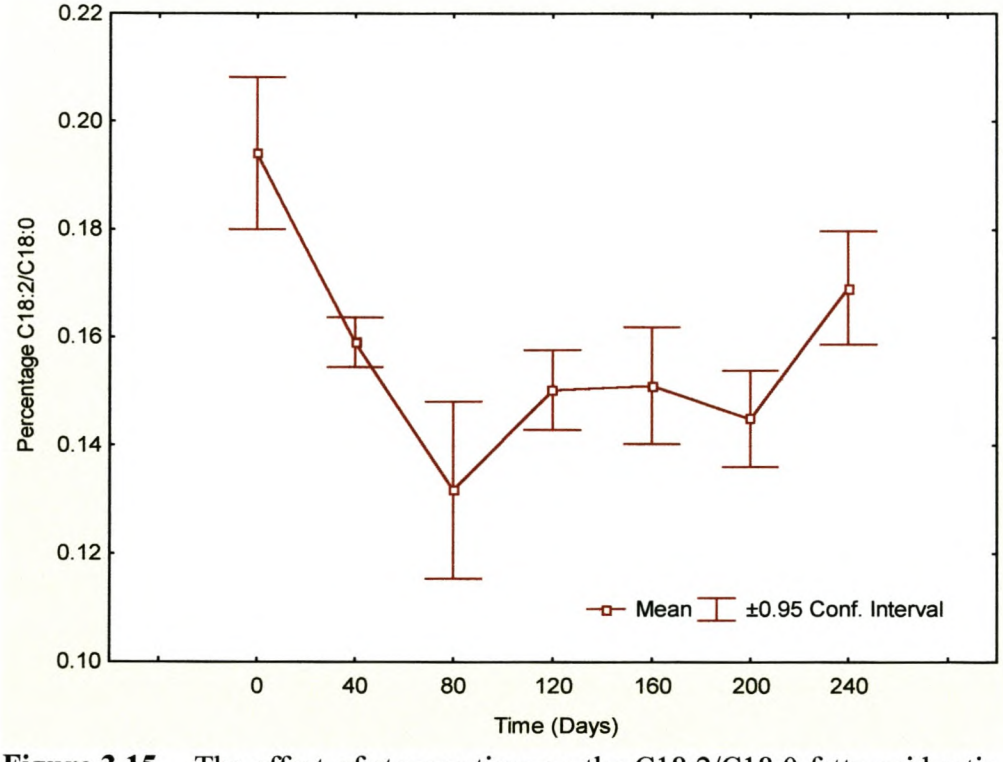


Figure 3.15. The effect of storage time on the C18:2/C18:0 fatty acid ratio of mango kernel fat (MKF).

$$16\,\text{ml} \times 0.916\,\text{g.ml}^{-1} = 14.656\,\text{g trioleic acid in the oil} \quad \ldots 3.1$$

Furthermore, the molecular weight of oxygen (O_2) is 32 g.mol^{-1} and 3 molecules of this is required to react completely with trioleic acid.

$$3 \times 32\,\text{g.mol}^{-1} \times \frac{14.656\,\text{g}}{885.5\,\text{g.mol}^{-1}} = 1.589\,\text{g}\,O_2 \text{ required } \ldots \quad \ldots 3.2$$

$$\frac{1.8\,\text{ml}}{22\,400\,\text{ml}} \times 32\,\text{g} = 0.0257\,\text{g}\,O_2 \text{ available in the headspace} \quad \ldots 3.3$$

Therefore only 4.85% of the required oxygen was available to oxidise a single double bond, or similarly, to oxidise all three oleic acid moieties, only 1.62% of the required level would be available.

Conclusion

The fatty acid composition of mango kernel fat obtained from this study was compared to that reported in previous studies. The oxidative stability of mango kernel fat correlated well with the predictions made before commencement of the shelf life study (expected high stability against oxidation due to the high level of unsaturation of the MKF) when the tocopherol content and fatty acid profile of MKF were taken into consideration.

The polyunsaturated fatty acids (C18:2 & C18:3) decreased, while saturated fatty acids (C16:0 & C18:0) and linoleic (C18:1 *cis*) acid exhibited the expected stability over a period of 240 days. The C18:2/16:0 and C18:2/18:0 ratios indicated decreases over 240 days, which is characteristic of the oxidation process in oils. Oxidative deterioration of mango kernel fat was therefore successfully monitored by analysing the changes in relative percentages of fatty acids present in the fat, using gas chromatography. Although this method is time consuming, it can be highly recommended for analysing the oxidative status of vegetable oils and fats.

The different temperatures used (5, 15, 25 and 40°C) had a limited effect on the fatty acid profiles. The limited amount of oxygen in contact with the oil, also

showed similar results with those of the samples stored without oxygen and it is recommended that higher concentrations of oxygen be used in future studies. It is also recommended that the evaluation of oxidative stability be done with more than one method to allow determination of the different stages occurring during the oxidation process.

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CHAPTER 4

DETERMINATION OF THE OXIDATIVE STABILITY OF MANGO (*Mangifera indica* L.) KERNEL FAT DURING STORAGE USING THE PEROXIDE VALUE, CONJUGATED DIENE VALUE AND *p*-ANISIDINE VALUE TESTS

indica L. seed is enclosed by a rather woody to leathery endocarp and contains 9 – 13% fat (Moharram & Moustafa 1982; Puravankara *et al.*, 1999).

The fatty acid profile of mango kernel fat has indicated a high content of stearic (C18:0) and oleic (C18:1) acids (Baliga & Shitole, 1981; Van Pee *et al.*, 1981; Narasimha Char & Azeemoddin, 1988). The remaining fatty acids are, in increasing order of chainlength, palmitic (C16:0), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acid (Van Pee *et al.*, 1981). The ratio of saturated to unsaturated fatty acids is approximately 40:60 (Moharram & Moustafa, 1981).

One of the main factors influencing the oxidative stability of fats and oils is the fatty acid profile, as higher saturation is directly proportional to chemical stability (Gunstone, 1996). From the view of fat oxidation in foods, the important lipids are those containing unsaturated fatty acids, particularly oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) (White, 1995). The final malodorous breakdown product of oleic acid is nonanal, while linoleic and linolenic acids break down to form 2,4-dienal and 2-alkenal (Crapiste *et al.*, 1999).

Lipid oxidation is one of the major causes of food spoilage and is of great economic concern to the food industry, as it leads to the development of off-odours and decreases the nutritional value of food (Nawar, 1996). In order to produce a healthier product for the consumer, the shelf life or oxidative stability of vegetable fats is the top priority for the oil manufacturer (Gunstone, 1996).

Vegetable oils generally contain natural antioxidants, which are extracted along with the oil; however, their level may be reduced during refinement (Gunstone, 1996). These compounds are usually tocols, i.e. tocopherols and tocotrienols. Phenolic antioxidants (especially flavonoids), which occur naturally in plants, are also recognised as important compounds in conferring stability against autoxidation of vegetable oils (Khan & Shahidi, 2000). Tannins and flavones have been detected in the mango kernel and the total polyphenol content has been reported as 79.5% (Kabuki *et al.*, 2000).

It is generally agreed that the reaction with molecular oxygen and the subjection to elevated temperatures are the main factors influencing the oxidative deterioration of lipids (Nawar, 1996).

Methods used to measure primary oxidation include the peroxide value (PV) and conjugated diene (CD) tests (Marsili, 1993). The PV test determines all substances, in terms of mille equivalents of peroxide per kilogram of sample, which

oxidise potassium iodide under the conditions of the test (Nawar, 1996). The maximum PV reached tends to decrease with the temperature of oxidation, suggesting that the activation energy for the decomposition reaction is higher than that for the production of hydroperoxides (Crapiste *et al.*, 1999). Narasimhan *et al.* (2001) reported increased peroxide values in thermally stressed refined palm oil. Sunflower oil also showed an elevated PV at higher temperatures (Maloba *et al.*, 1996). Freshly-refined material should have a peroxide value below one and is perceived as rancid at a peroxide level of about ten (Gunstone, 1996). The CD value is expressed as a percentage of conjugated dienoic acid in the oil and its value correlates fairly well with the PV (White, 1995). One of the first steps in the oxidation of linoleic acid (C18:2) or higher polyunsaturated fatty acids (PUFA) in oil is a shift in the position of the double bonds (White, 1995). The greater the amount of PUFA in the oil, the greater the potential increase in conjugated diene formation. Conjugated diene values of up to 6% are expected for high PUFA oils (Gunstone, 1996).

Secondary oxidation products can be measured by the *p*-anisidine value (AV) procedure, which is simple, reproducible and quick (White, 1995). The AV procedure was developed to monitor the reaction of α- and β-aldehydes (mostly 2-alkenals) with *p*-anisidine reagent (Gunstone, 1996). The *p*-anisidine value generally has a strong relationship to the results of headspace volatile analysis (for all selected aldehydes but nonanal) and the sensory evaluation of overall odour intensity (Tompkins & Perkins, 1999). A highly significant correlation is also usually observed between polymer content (polymerised acylglycerols) and *p*-anisidine values (Crapiste *et al.*, 1999). Well-refined oils generally have *p*-anisidine values ranging between 1.0 and 10.0 $mmol.kg^{-1}$ fat.

In this study, the influence of oxygen and temperature on the oxidative properties of mango kernel fat was determined on samples subjected to a range of temperatures and exposed to a limited amount of oxygen or the exclusion of oxygen. The state of oxidation was measured every 40 days for 240 days by the peroxide value, conjugated diene value and *p*-anisidine value tests.

Materials and methods

Three individual batches crude, cold-pressed, mango kernel fat (MKF) were obtained from Specialised Oil cc. (Industria road 2, Industria, Louis Trichardt 0920, South

Africa). The clean mango stones were collected from a fruit juice plant (Valley Farms, P.O. Box 163, Levubu 0929, South Africa), followed by manual decortication using a knife. The mango kernels were dried in an electronically controlled oven for 24 hours at 60°C until a moisture content of 12.5% was reached. The dried kernels were then stored at room temperature for three to six days and mechanically pressed at 45°C. The fat was not refined or bleached.

The MKF was stored in 25 ml Chromacol clear glass bottles. Prior to filling, the oil was liquefied at 45°C. This was done to ensure the liquid state of the fat during the entire filling time of the bottles before re-solidification of the fat occurred. Headspace was allowed in 50% of the samples by filling the bottles with approximately 16 ml of the fat. The other half of the samples were filled to the top with fat and closed with a Teflon cap to ensure total oxygen exclusion.

Samples were stored in the dark at 5°C, 15°C, 25°C and 40°C for 240 days. Adequate vials for every oil sample were subjected to each storage condition so that no vial had to be reused once it had been removed from storage and used for analyses.

At 40-day intervals, samples from all three batches were liquefied for 10 minutes at a temperature of *ca.* 45°C. This was necessary as MKF was still solidified at 25°C. This led to an easily obtainable, homogenous liquid sample. The samples were shaken lightly and opened just before analysis. Analyses were done in duplicate for all batches.

The peroxide value was determined according to the American Oil Chemists Society (AOCS) Method Cd 8 53 (1985). Sample sizes were, however, reduced to 0.5g ± 0.0009, using 100 ml instead of 250 ml Erlenmeyer flasks. Volumes were reduced to 10 ml of the acetic acid-chloroform solution, 10 ml of distilled water and a 0.01 M sodium thiosulfate titrant. The conjugated diene value (CD) was determined according to the AOCS Method Ti la-64 (1993). Sample sizes were reduced to 18 ml, diluting to volume with hexane in a 25 ml volumetric flask. The p-anisidine value was determined according to the AOCS Method Cd 18-90 (1992). Purification of the *p*-anisidine crystals was done in accordance with specifications obtained from Method CD 18-90. The purified crystals are depicted in Figure 4.1. The absorbances at 233 nm for CD and at 350 nm for AV were measured with a Phillips PU 8700 series, UV/Visible spectrophotometer (Phillips Scientific and Analytical Equipment). The total oxidation (TOTOX) value was calculated by the formula: TOTOX = 2PV +AV.

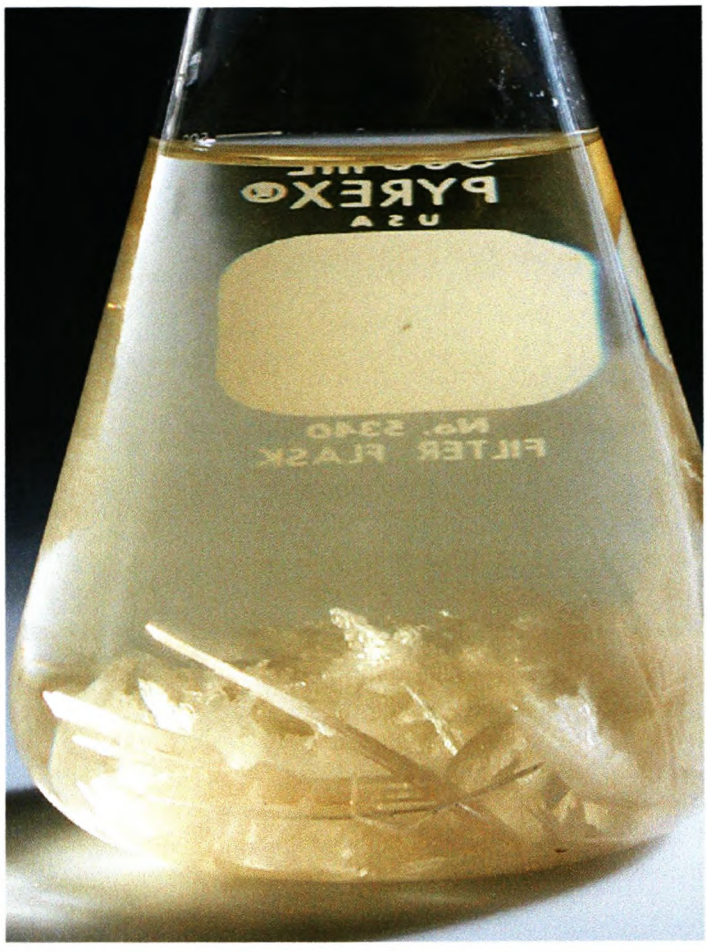

Figure 4.1 Purified *p*-anisidine crystals, photographed 12 hours after re-crystallisation.

The vitamin E content was determined using the high performance liquid chromatography (HPLC) method for the routine determination of tocopherols in animal feed and human foodstuffs (Manz & Phillipp, 1981). Analysis was performed by ARC – Animal Nutrition and Animal Products Institute, Private Bag X2, Irene, 0062.

Statistical analysis

Graphs were compiled using Statistica version 6. Every point on the graph indicates the average value calculated from two duplicates in three batches (six values). The bar around the average represents the 95% confidence interval for the average. The inspection of the overlapping of the 95% confidence intervals identified significant changes. When no overlapping occurred, significant differences were assumed.

Results and discussion

Peroxide value analysis

The freshly pressed MKF had an average PV of 2.7 meq.kg^{-1}, which increased over a period of time as hydroperoxides were formed. This initial increase up to 40 days in the samples unexposed to oxygen (Figure 4.2) and exposed to oxygen (Figure 4.3), changed direction after 40 days, indicating the breakdown of hydroperoxides. The PV of samples without headspace at 5°C showed a very slight increase in peroxide value from days 0 to 40 (Figure 4.2), whilst the higher temperatures (15, 25 and 40°C) led to greater increases. The same trend was observed for the samples stored with a limited amount of oxygen (Figure 4.3), namely higher maximum peroxide values for samples stored at 15, 25 and 40°C, and lower PV for samples stored at 5°C from days 0 to 40.

It is generally agreed that the reaction with molecular oxygen is the main reaction implicated in the oxidative deterioration of lipids (Nawar, 1996). This is evident, as the peroxide value of MKF is constantly higher in samples stored with headspace (Figures 4.4 & 4.5) in comparison with those stored with exclusion of oxygen. The rate of oxidation is also influenced by the surface area exposed to oxygen, explaining the minor oxidation (low peroxide values) observed for MKF with only a limited amount of oxygen included in the narrow, sealed 25 ml bottles. The samples were not shaken during storage, thus minimising the surface area between the

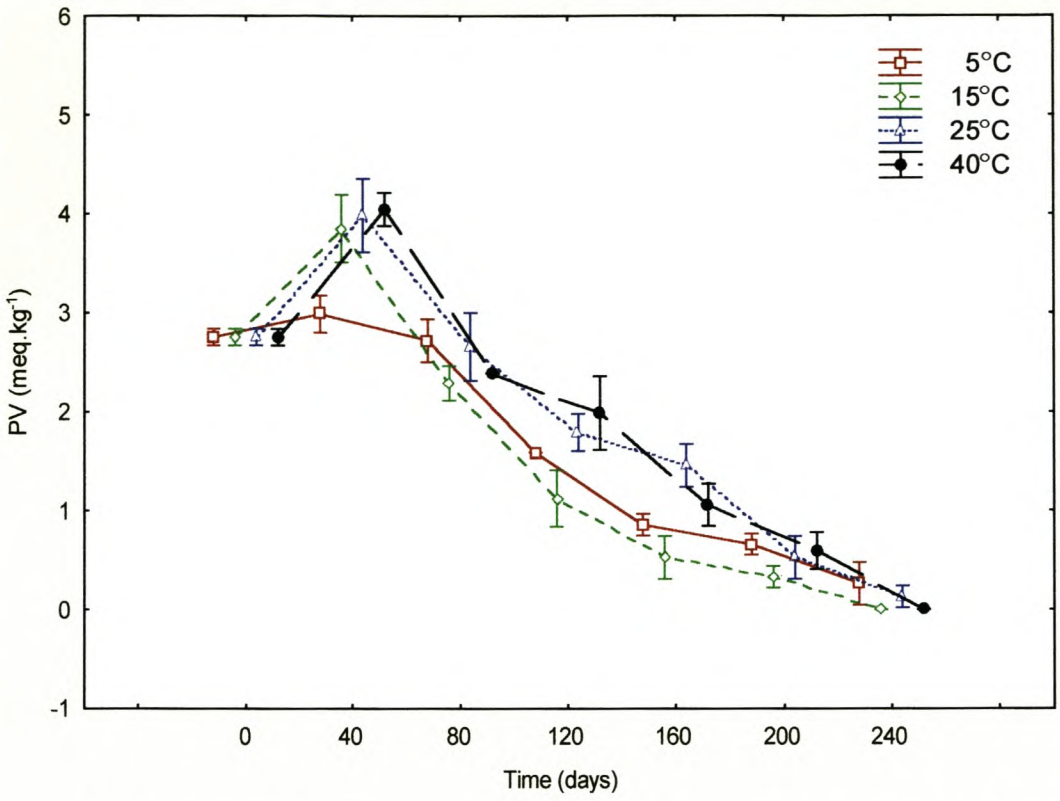

Figure 4.2. Changes in the peroxide value of mango kernel fat stored for 240 days at different temperatures with no oxygen present.

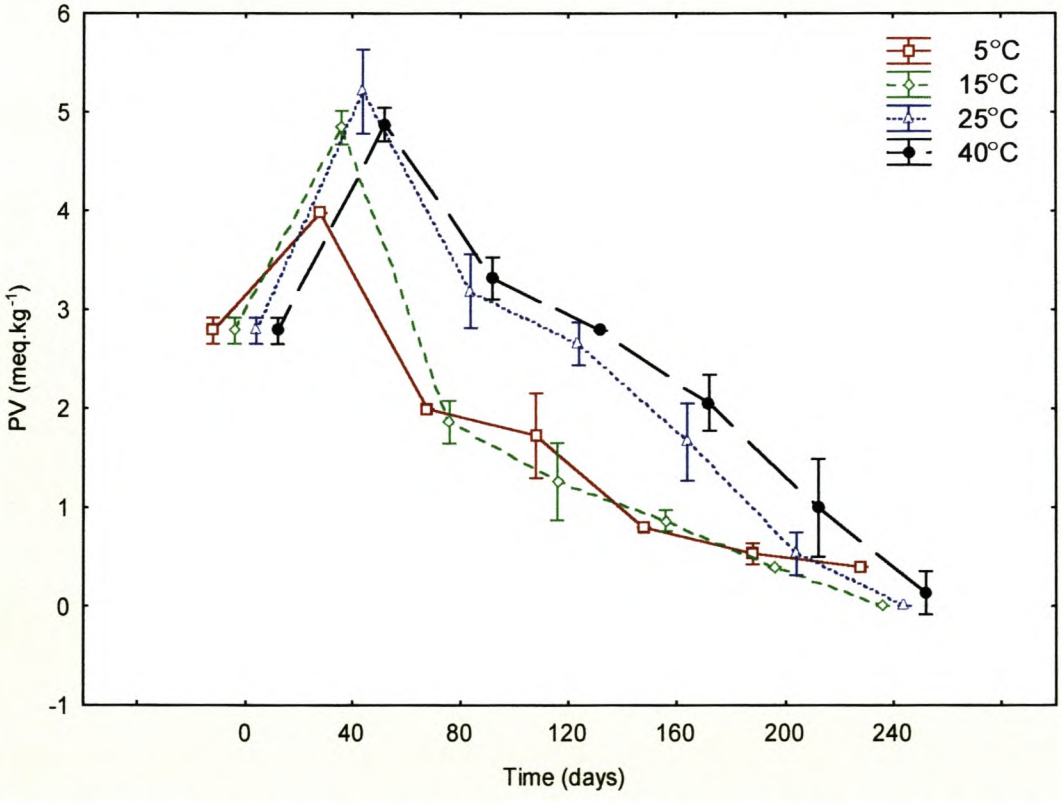

Figure 4.3. Changes in the peroxide value of mango kernel fat stored for 240 days at different temperatures with a limited amount of oxygen present.

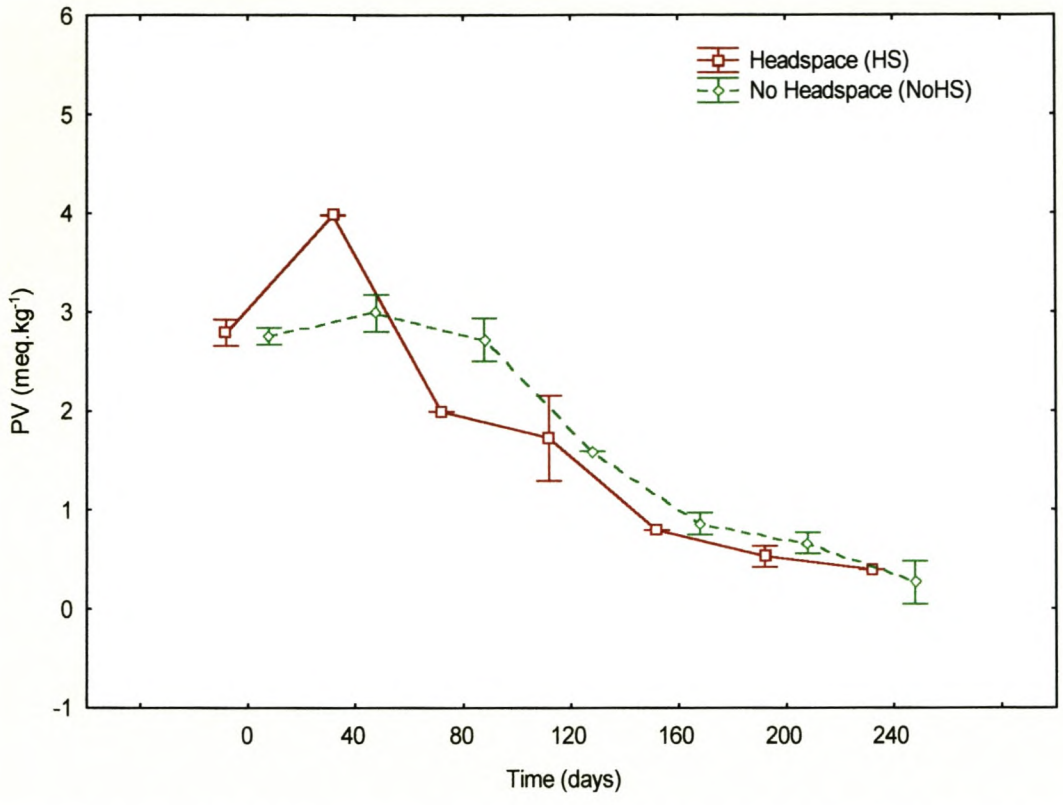

Figure 4.4. Peroxide values of mango kernel fat stored with and without headspace at 5˚C for 240 days.

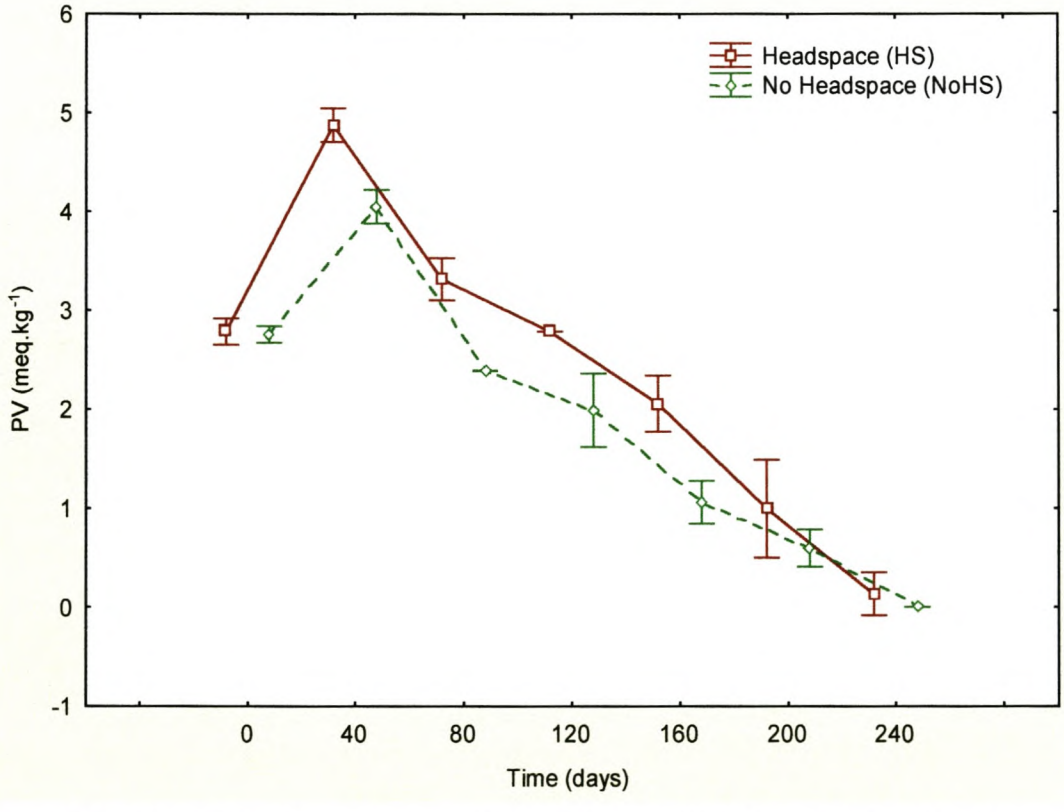

Figure 4.5. Peroxide values of mango kernel fat stored with and without headspace at 40˚C for 240 days.

fat and oxygen. Another possibility is that depletion of oxygen in the MKF samples with headspace took place between 40 and 80 days within every sample, as peroxide production ceased at this point.

The PV never exceeded five units for samples exposed to either set of oxygen conditions. It can therefore be assumed that the MKF was not rancid (as indicated by the PV) after a storage period of 240 days at temperatures of 5, 15, 25 or 40°C with a limited amount of oxygen available. Initial peroxide values of 2.7 meq.kg^{-1} were higher than the expected peroxide value of 1.95 – 1.99 meq.kg^{-1} (Joseph, 1995) for a freshly pressed product. This can be explained by the fact that the values were determined after liquefying the oil, which could explain the early production of hydroperoxides. In this respect the storage of the kernels before extraction of the oil must also be taken into account as the exact conditions were unknown.

The main fatty acids constituting the fatty acid profile of mango kernel fat are stearic (C18:0) and oleic (C18:1) acid (Narasimha Char & Azeemoddin, 1988). These fatty acids, especially oleic acid, are considered relatively stable against oxidative deterioration. The limited amount of oxygen in contact with the polyunsaturated fatty acids present in MKF (C18:2, 5%; C18:3, trace) led to minimal production of hydroperoxides and consequently, minor oxidation of the oil. This primary oxidation was concluded at 40 days for MKF samples at all temperatures and under both oxygen conditions. The oxidative stability of MKF was probably also improved by the inhibitory effect of the phenolic compounds present in MKF (Satue *et al.*, 1995; Kabuki *et al.*, 2000). Antioxidants are more readily depleted at higher temperatures, leading to greater and more prolonged activity at lower temperatures by suppressing the oxidative chain for longer.

The main component responsible for oxidative stability in edible oils is the tocopherol content of the oils (Chu & Kung, 1998). The high α-tocopherol content determined for MKF in this study (100 mg.100g^{-1}) could also contribute to the stability of the fat, although α-tocopherol has shown pro-oxidant activity at high concentrations (>500 ppm = 50 mg.100 g^{-1}) in various cases (Koskas *et al.*, 1984; Satue *et al.*, 1995). It has, however, been proved that the effectivity of α-tocopherol increases with temperature (Marinova & Yanishlieva, 1992).

Conjugated diene value analysis

Very low conjugated diene values ranging from 0.05% to 0.18% could be observed for MKF at all temperatures, both for samples where oxygen was excluded (Figure 4.6) and for those where oxygen was included (Figure 4.7). This corresponded with the low polyunsaturated fatty acid (PUFA) profile of MKF and the CD values of up to 6% expected for high PUFA oils (Gunstone, 1996).

The conjugated diene value of MKF at day 0 was 0.07%. This value increased significantly for all samples at day 40, indicating the shifting in double bonds of the polyunsaturated fatty acids during the first steps of oxidation. This increase was consistently higher for samples exposed to oxygen (Figures 4.8 & 4.9), especially the samples stored at 40°C at day 240. Once the dienes were in the stable conjugated form after day 40, the values stayed constant for samples stored without oxygen (Figure 4.6), except for a slight increase at day 160, which can be explained by the higher content of unsaturated fatty acids in the specific samples. As the triacylglycerols of butters consist mainly of monounsaturated and saturated fatty acids and only 6% polyunsaturated acids and the MKF was repeatedly solidified and liquefied during preparation for storage, it can be argued that the homogenous nature of the fat was disturbed at times, explaining the slight variation in the values. This variation was more defined for the samples stored with limited exposure to oxygen (Figure 4.7), especially for samples stored at 40°C, indicating further shifting in double bonds due to this elevated temperature. The elevated oxygen levels affecting the structural changes of the polyunsaturated fatty acids present in these samples led to elevated levels of conjugated dienes. There was no difference in the range of conjugated diene values obtained at different temperatures when exposed to oxygen and the variation within theses ranges can be ascribed to the differences in polyunsaturated fatty acid content of the respective samples.

p-Anisidine value analysis

The *p*-anisidine values of MKF showed a great deal of variation between day 0 and day 240 for samples unexposed (Figure 4.10) and exposed (Figure 4.11) to oxygen. Initial values of 2.2 $mmol.kg^{-1}$ correlated well with those of fresh low linoleic soybean oil (AV = 1.52 $mmol.kg^{-1}$) and sunflower oil (AV = 0.96 $mmol.kg^{-1}$) (Tompkins & Perkins, 1999; Crapiste *et al.*, 1999).

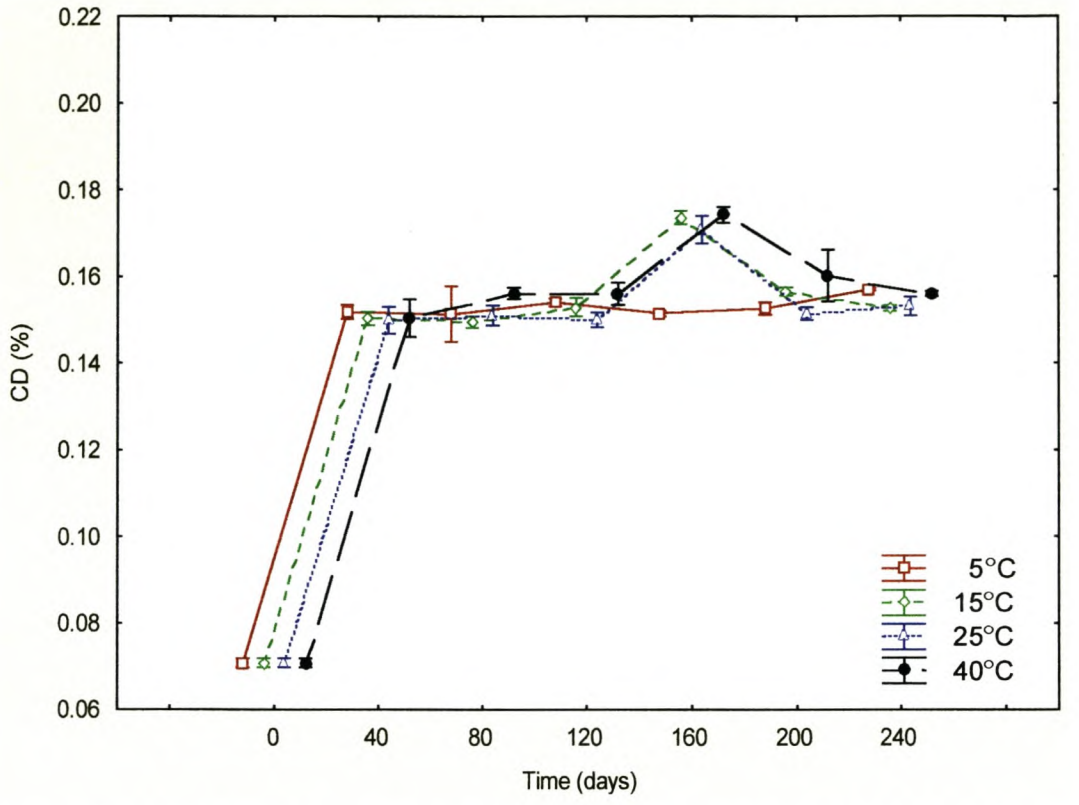

Figure 4.6. Changes in the conjugated diene value of mango kernel fat stored for 240 days at different temperatures, with no oxygen present.

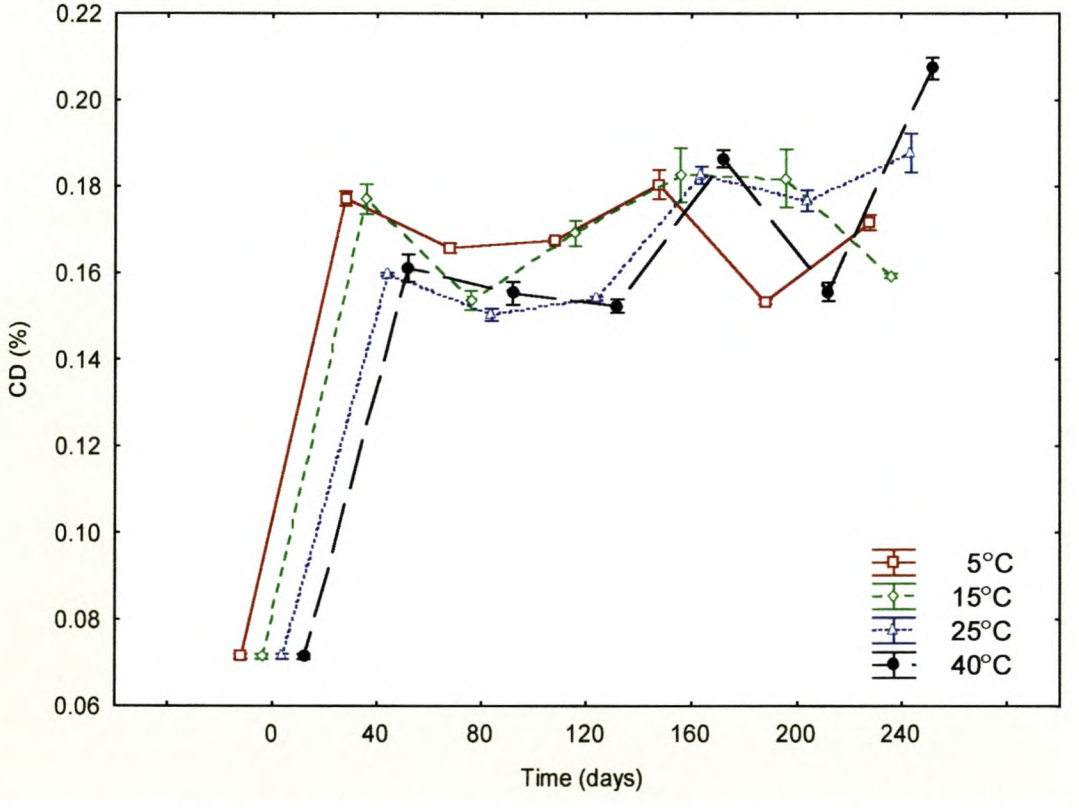

Figure 4.7. Changes in the conjugated diene value of mango kernel fat stored for 240 days at different temperatures, with a limited amount of oxygen present.

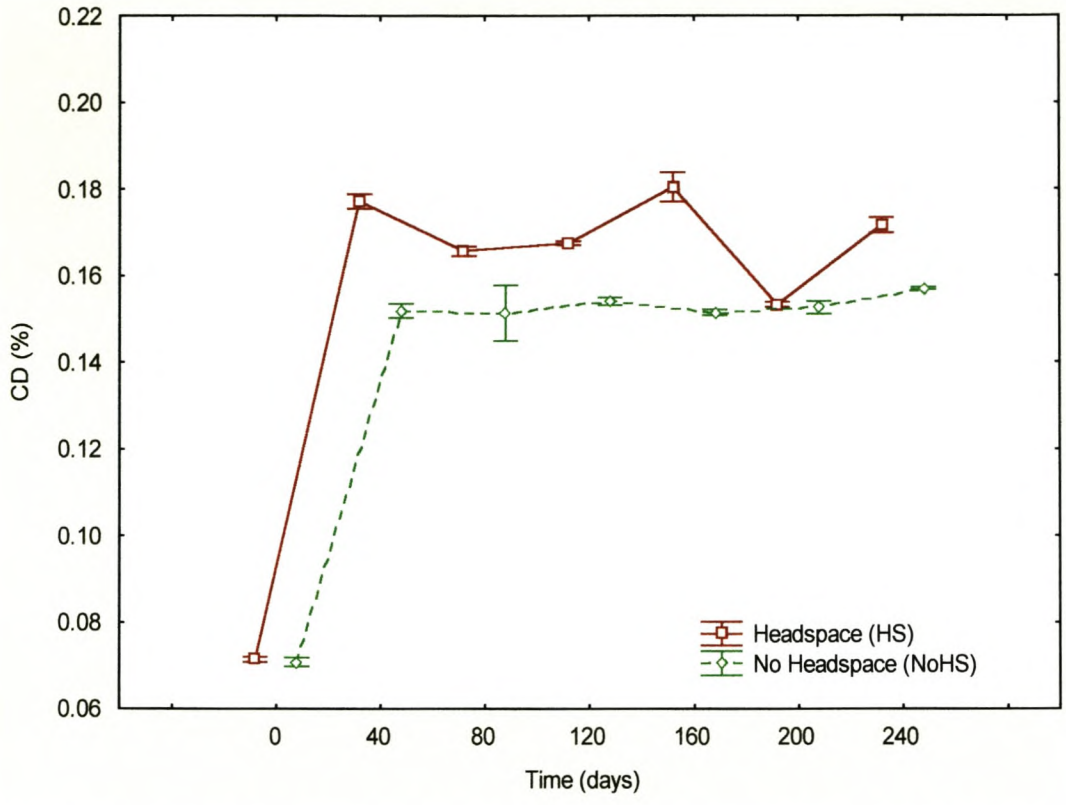

Figure 4.8. Conjugated diene values of mango kernel fat stored with and without headspace at 5°C for 240 days.

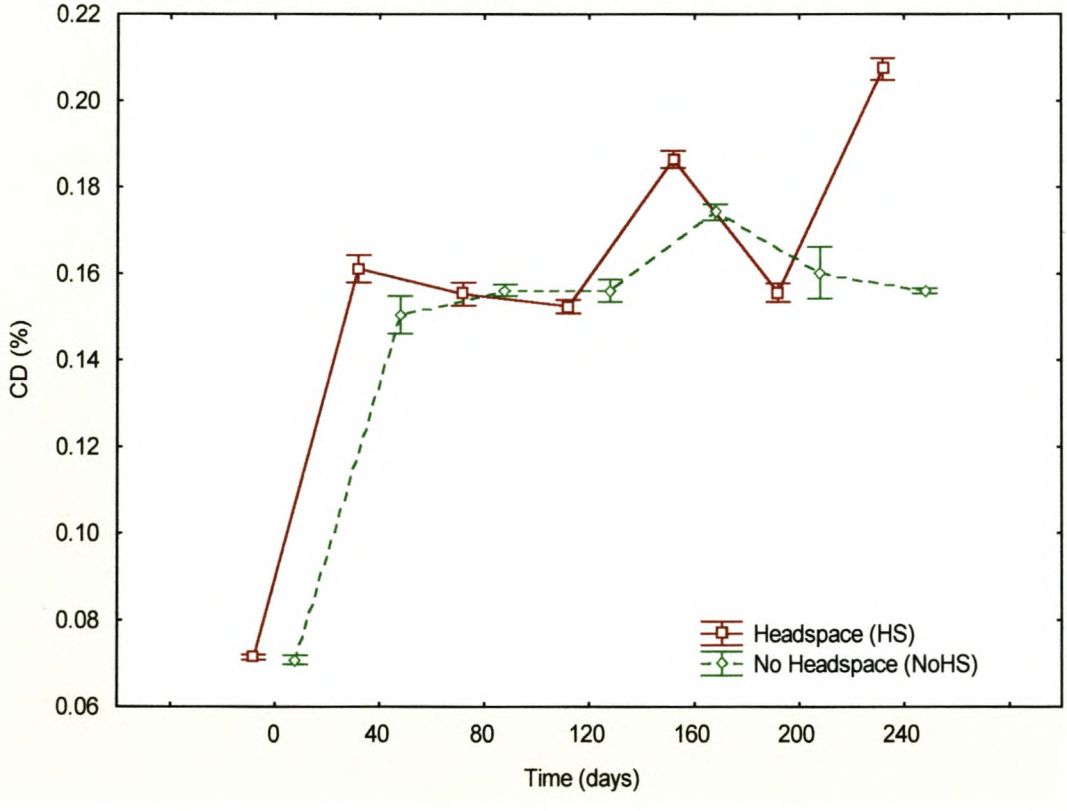

Figure 4.9. Conjugated diene values of mango kernel fat stored with and without headspace at 40°C for 240 days.

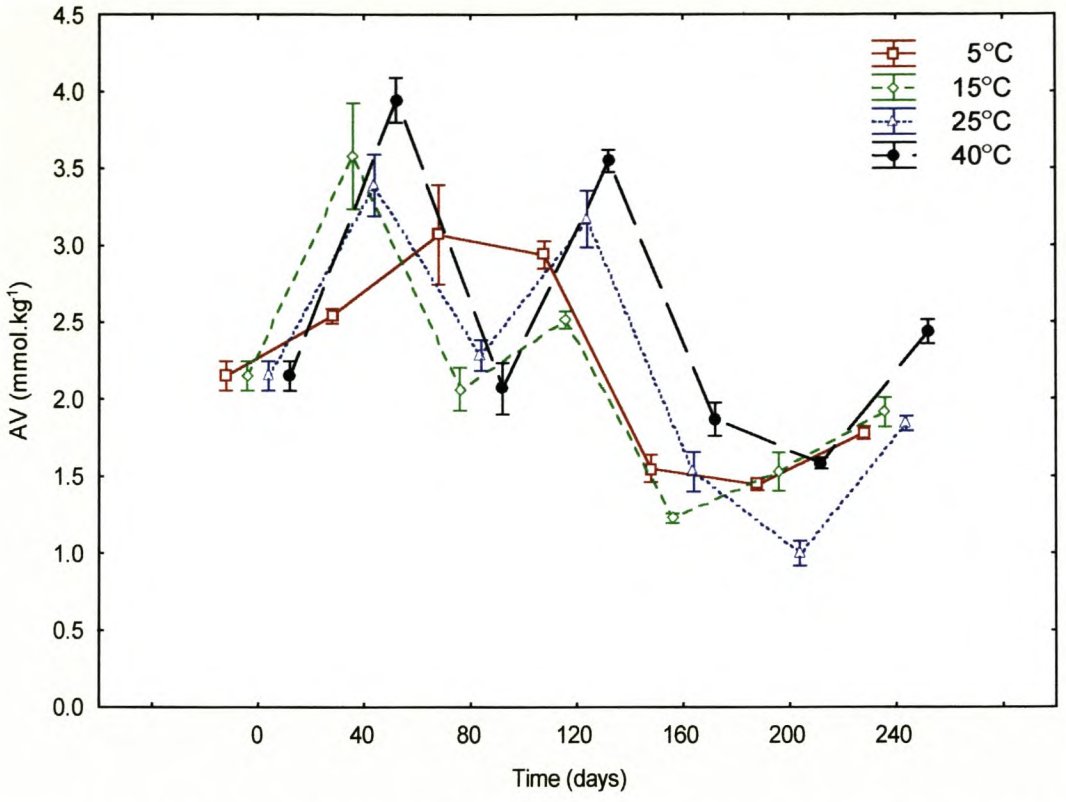

Figure 4.10. Changes in the *p*-anisidine value of mango kernel fat stored for 240 days at different temperatures, with no oxygen present.

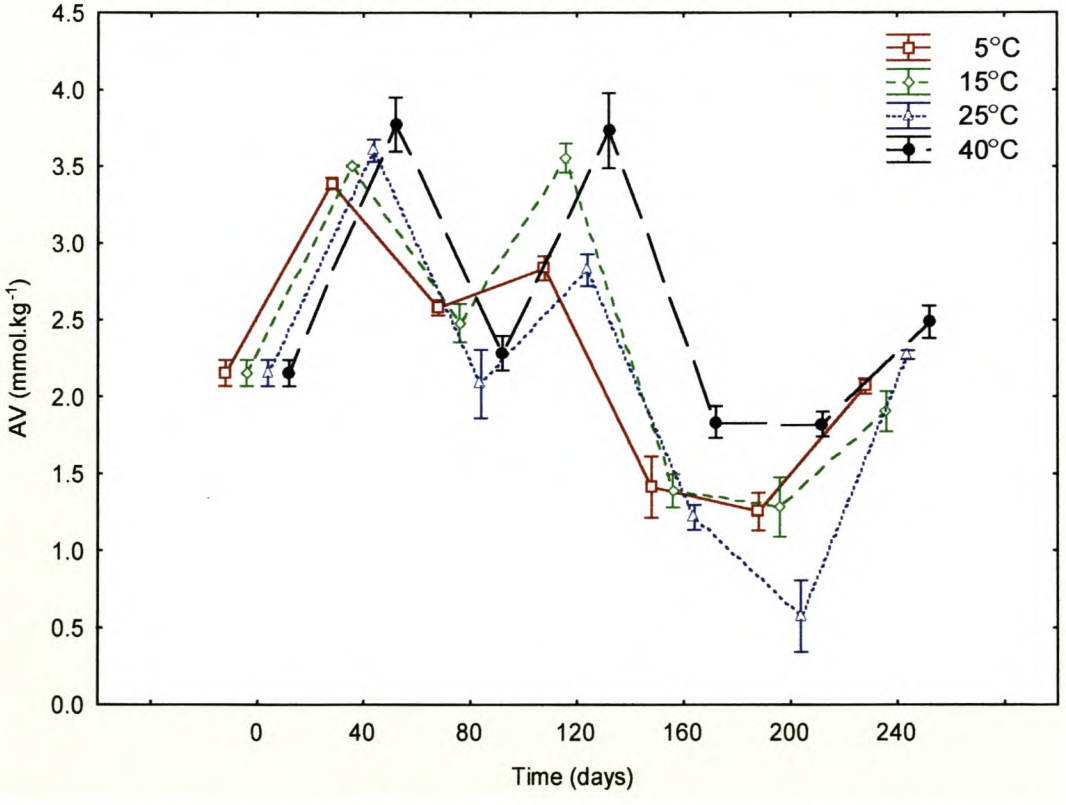

Figure 4.11. Changes in the *p*-anisidine value of mango kernel fat stored for 240 days at different temperatures, with a limited amount of oxygen present.

MKF showed an extremely low range of *p*-anisidine values, of between 1 and 4 $mmol.kg^{-1}$ after storage at 40°C for 240 days, in comparison to sunflower oil after 60 days at 47°C (AV = 45 $mmol.kg^{-1}$) (Crapiste *et al.*, 1999).

Samples with and without headspace showed almost identical trends (Figures 4.12 & 4.13). The sample stored without oxygen at 5°C did not reach the same AV as the sample stored with oxygen and took longer before reaching the maximum AV (Figure 4.12). This can be related to the PV at 5°C, where the samples without headspace do not show a significant breakdown of hydroperoxides before 80 days (Figure 4.4). Secondary products therefore will not be detected at an early stage. At 40 °C the samples stored with and without headspace, showed similar trends and values.

All the *p*-anisidine values decreased significantly after 160 days, which can be explained by the fact that most of the hydroperoxides produced during the primary stages of oxidation had already been broken down to secondary products at that stage in the oxidative process.

The AV expresses the secondary products formed during the oxidation of unsaturated fatty acids, which are measured as 2,4-dienal and 2-alkenal. The nonanal formed by the breakdown of oleic acid cannot be expressed by the AV. This explains the low *p*-anisidine values for mango kernel fat, as the oleic acid is by far the more pronounced fatty acid in MKF (Narasimha Char & Azeemoddin, 1988). When considering the fatty acid profile of MKF, the range of AV values seemed agreeable, but the non-linear nature of the graph suggests the need for more research, especially concerning the polymerisation reactions likely to occur due to the production of highly reactive free radicals. The determination of the polar fraction distribution, constituted by oxidized triacylglycerides, in the MKF might not only be a good reference to the non-linear AV readings obtained, but possibly will be able to indicate the origin of deterioration (Crapiste *et al.*, 1999).

Total oxidation (TOTOX)

The TOTOX value for MKF increased up to day 40, after which it decreased again until day 240. The same trend could be seen for samples stored without headspace (Figure 4.14) and with headspace (Figures 4.15). The depletion of oxygen was apparent when total oxidation decreased from day 40. For all the samples stored with

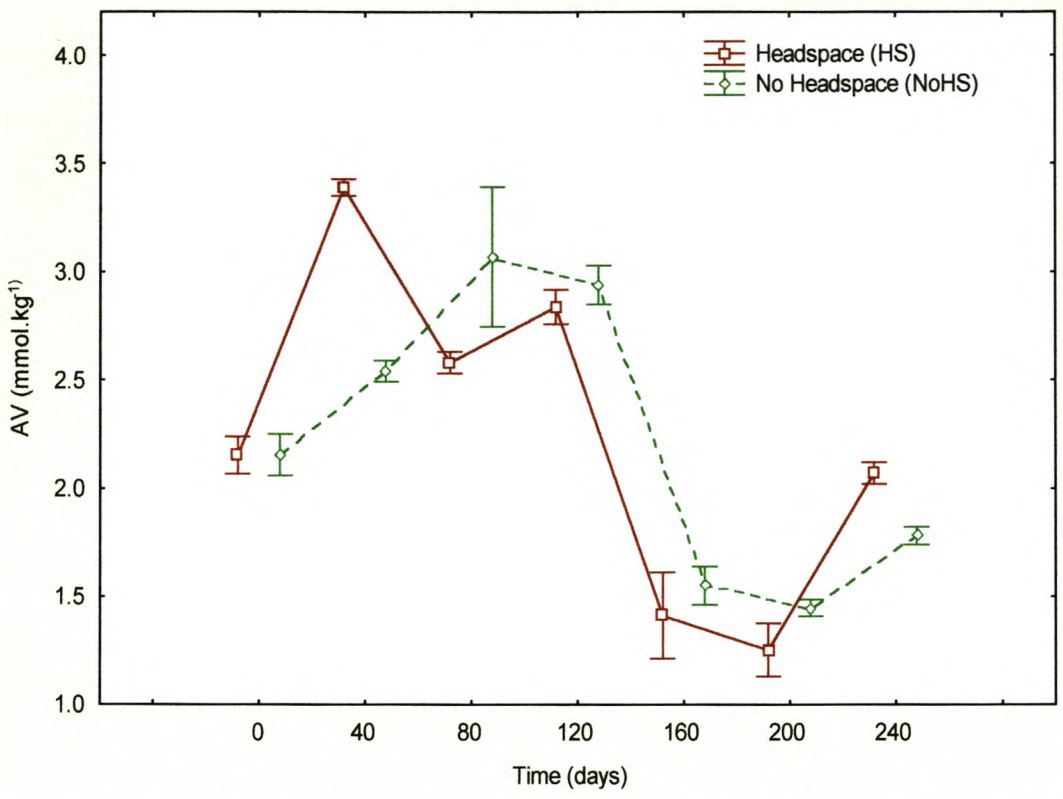

Figure 4.12. The *p*-anisidine values of mango kernel fat stored with and without headspace at 5°C for 240 days.

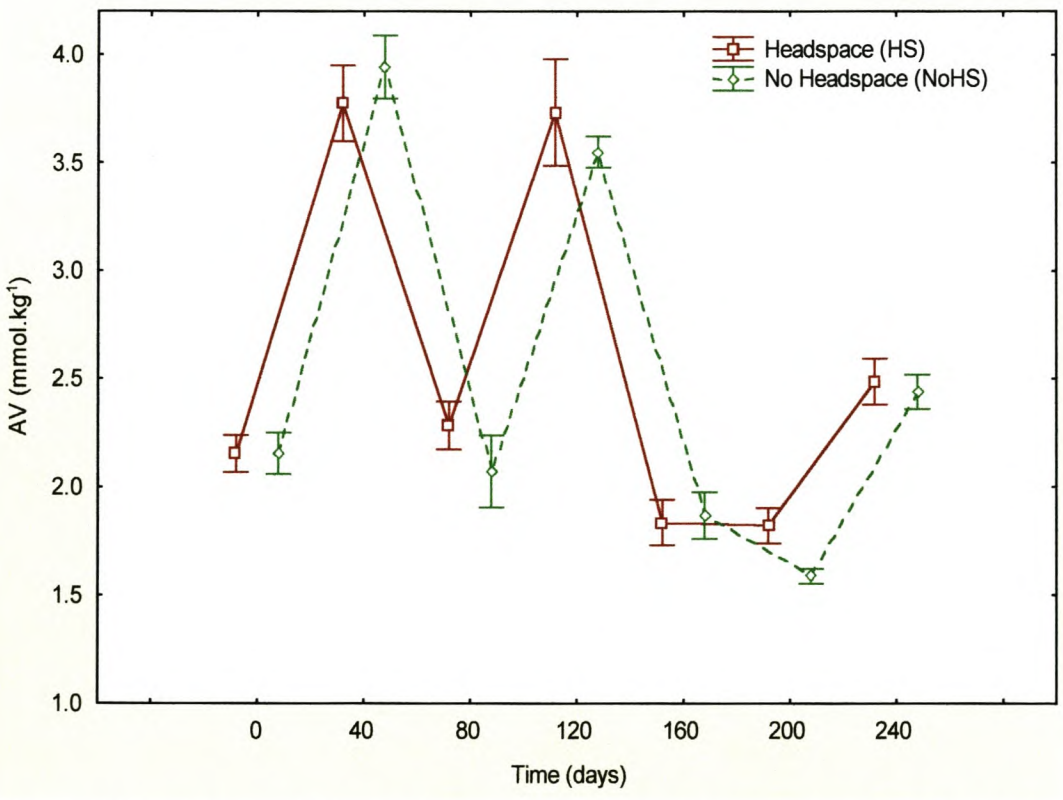

Figure 4.13. The *p*-anisidine values of mango kernel fat stored with and without headspace at 40°C for 240 days

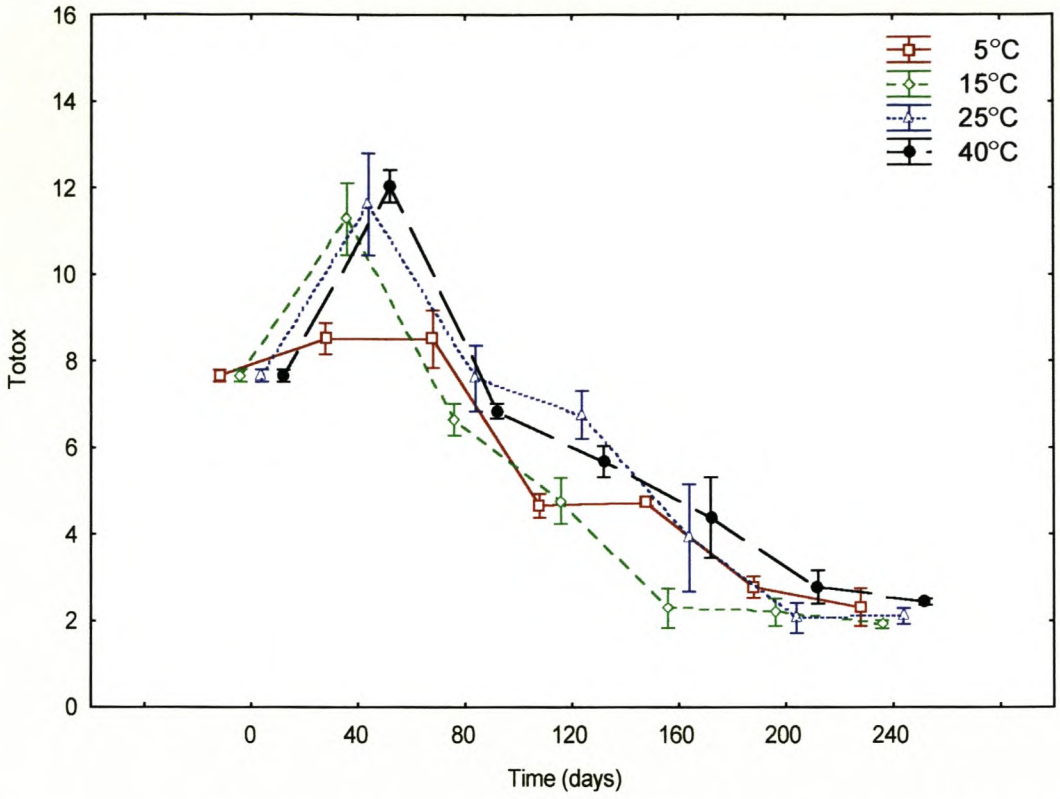

Figure 4.14. Changes in total oxidation of mango kernel fat stored for 240 days at different temperatures, with no oxygen present.

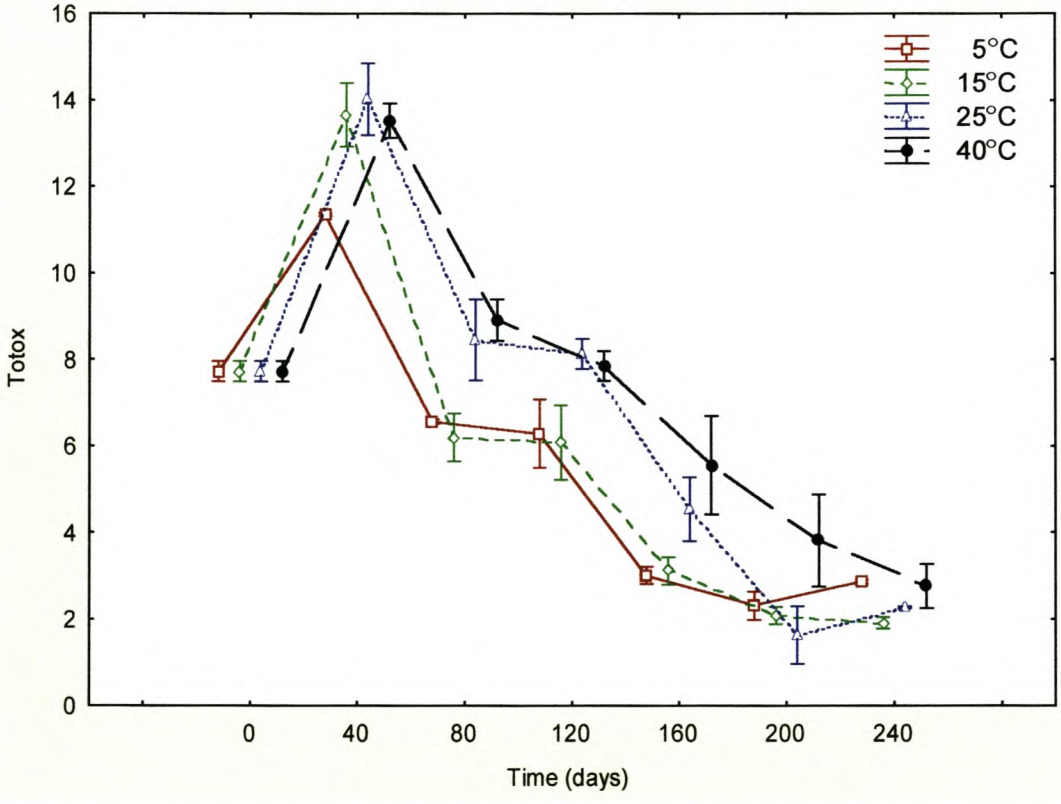

Figure 4.15. Changes in total oxidation of mango kernel fat stored for 240 days at different temperatures, with a limited amount of oxygen present.

headspace, a delay in oxidation was observed between days 80 and 120. It is clear that an increase in AV was observed for all samples exposed to a limited amount of oxygen on day 120, which will influence the total oxidation value positively. The difference in total oxidation of samples stored with and without oxygen also clearly indicated the positive influence of a limited amount of oxygen on the deterioration of the lipids (Figures 4.16 & 4.17). The detrimental effect of heat on oxidation was also evident as higher total oxidation levels were reached for samples stored at higher temperatures (Figures 4.14 & 4.15).

Conclusion

The results confirmed the expected influence of storage temperature and oxygen availability on crude mango kernel fat. Relatively low or no oxidation occurred at lower temperatures with exposure to limited oxygen levels. Peroxide values did not exceed 5 meq.kg^{-1} after 240 days of storage at 5, 15, 25 or 40°C, indicating that the oil was very stable against oxidation and showed no sign of rancidity (according to peroxide values obtained for MKF). The polyunsaturated fatty acids, linoleic and linolenic acids, were also well preserved and a percentage was oxidised to a stable state during pre-heating and the first 40 days of storage as seen by the conjugated diene value test.

The AV values were not very high, due to the low linoleic acid content of MKF. A comparison of the PV and AV values clearly demonstrated the relationship between primary and secondary oxidation products, showing an increase in secondary products as the primary products were broken down. Since it is difficult to assess total oxidation, several analyses were needed. The TOTOX value gave an indication of declining oxidation in the limited oxygenated environment. Considering the results obtained from the analytical tests, mango kernel fat can be recommended for use in secondary products, as it can withstand the factors influencing oxidation very well.

The influence of oxygen can, however, be more thoroughly investigated by increasing the oxygen concentration in the oil sample or by incorporating shaking in the shelf life study of the fat to increase the surface area for oxidation uptake. Determination of the polar fraction constituted by the mono-, di- and polymers of triglyceride could be utilised to discover the origin or deterioration by evaluating the

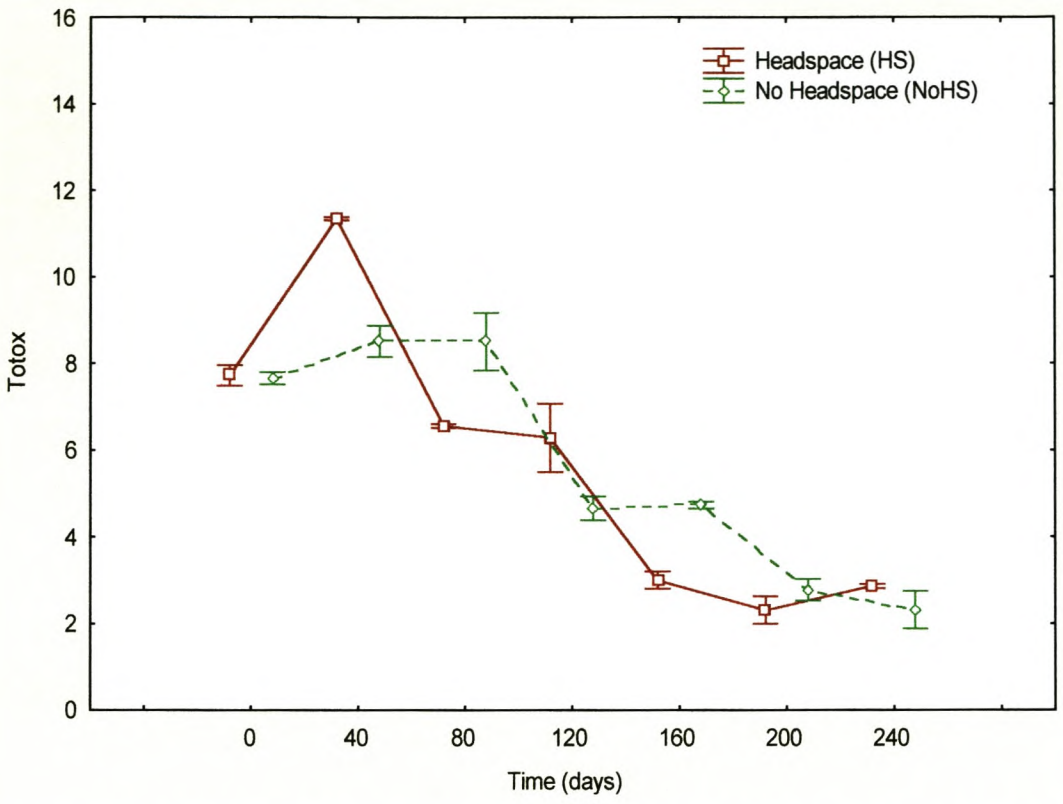

Figure 4.16. Total oxidation values of mango kernel fat stored with and without headspace at 5°C for 240 days.

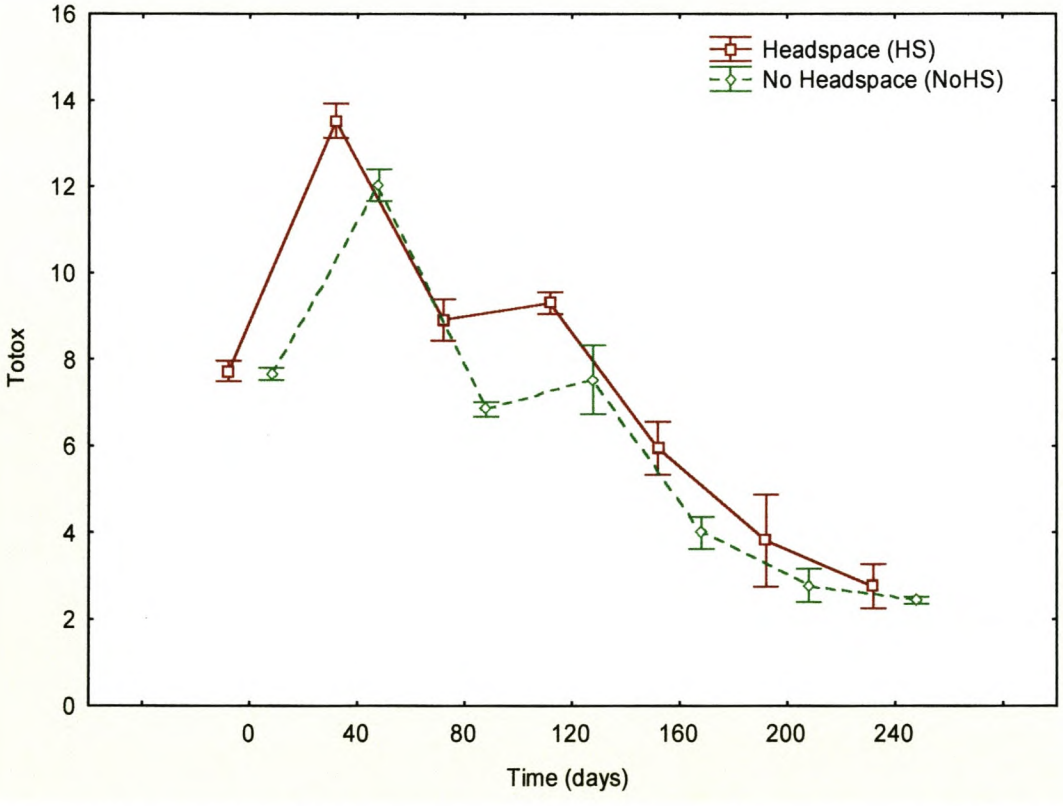

Figure 4.17. Total oxidation values of mango kernel fat stored with and without headspace at 40°C for 240 days.

polar compound distribution. In addition, the characterisation of the unsaponifiable compounds in the MKF will also be helpful in research concerning the oxidative stability.

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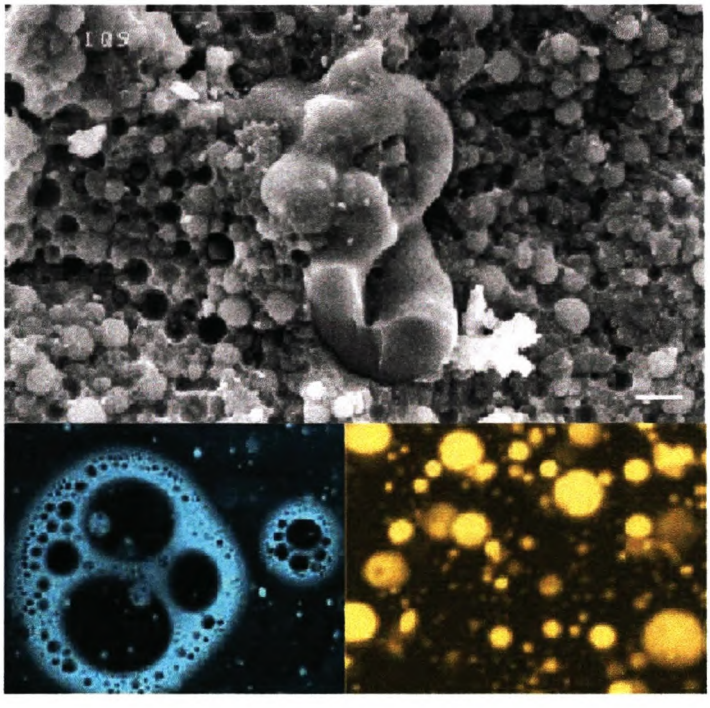
CHAPTER 5

THE EFFECT OF EMULSIFICATION ON THE OXIDATIVE STABILITY OF CRUDE, COLD PRESSED MANGO KERNEL FAT



Chapter 5

THE EFFECT OF EMULSIFICATION ON THE OXIDATIVE STABILITY OF CRUDE, COLD-PRESSED MANGO KERNEL FAT.

Summary

The effect of emulsification on the oxidative stability of crude, cold pressed mango kernel fat was studied during a shelf life trial by use of the peroxide value (PV), conjugated diene (CD) value and *p*-anisidine value tests. Water-in-oil emulsions of the mango kernel fat were made using soy lecithin and distilled monoglyceride as emulsifiers. Samples were stored at 5, 15, 25 and 40°C without exposure to UV light or oxygen. Oxidative deterioration was monitored over a period of 240 days during 40-day withdrawals. Values obtained were compared to those of the crude mango kernel fat. The only increase in PV for emulsified MKF was observed after 40 days of storage at 5°C (3.4 meq.kg^{-1}), while samples stored at 15, 25 and 40°C showed no increase in peroxide values. Peroxide values of emulsified and MKF samples stored at 5°C compared well with each other, while the difference in maximum values at higher temperatures was obvious (40°C: MKF = 4 meq.kg^{-1} vs. Emulsion = 2.7 meq.kg^{-1}). CD values increased with increasing storage temperatures 5°C (0.158%), 15°C (0.159%), 25°C (0.168%) and 40°C (0.178%). Values correlated well with those obtained for crude MKF. The AV for emulsified mango kernel fat showed excellent stability, especially for temperatures 5, 15, and 25°C, ranging mostly between 1.5 and 3 mmol.kg^{-1}. When comparing these values with those of the crude unemulsified MKF stored at the same temperatures, emulsification seemed to have a stabilising effect in all cases and the non-linear nature of the trends decreased. The most variation in AV for emulsified MKF was observed at 40°C with a value of 3.6 mmol.kg^{-1} at 40 days. At 40°C, however, the emulsified and unemulsified MKF samples showed a very similar profile to that of crude MKF and it could be assumed that emulsification had no influence at this temperature. Total oxidation (TOTOX) curves for emulsified MKF indicate similar maximum values (8 units) and trends for samples stored at 15, 25, and 40°C, while samples stored at 5°C reached higher total oxidation values (10 units). This was also the only storage temperature where emulsions reached higher total oxidation values (9.5) than the crude MKF (8.5).

Introduction

The susceptibility of lipids to oxidation is an eminent factor influencing the deterioration of raw and processed products (Gunstone, 1996). Lipid oxidation is responsible for changes in food products such as taste, odour, appearance, texture, shelf life and nutritional profile (Nawar, 1996). Foods with a high fat content are especially prone to oxidative deterioration when elevated levels of polyunsaturated fatty acids (PUFA) are present (White, 1995). This poses a lot of problems for the food industry, as the current health trend is moving away from the use of saturated fats to oils containing high levels of PUFA (Marsili, 1993). As lipids are usually distributed in structurally and compositionally heterogeneous matrices like emulsions, the control of lipid oxidation is often limited (McClements & Decker, 2000).

An emulsion can be defined as a fine and stable dispersion of one immiscible fluid in the other (Hoffmann, 1989). Emulsions are divided in either water-in-oil or oil-in-water emulsions. In a water-in-oil emulsion the water droplets are dispersed in a semi-solid fat phase containing fat crystals and/or liquid oil (Gunstone, 1996).

Oxidative stability in emulsions depend on the interplay between a number of factors including type and concentration of emulsifier, size of oil droplets, fatty acid composition of the oil, surface area of interface, viscosity of the aqueous phase, composition and porosity of the aqueous matrix, and pH (Nawar, 1996). The rate of lipid oxidation in emulsions may be limited by the speed that free radicals, hydroperoxides or lipids can diffuse from one region to another within a droplet (McClements & Decker, 2001). In oil-in-water emulsions, or in foods where oil droplets are dispersed into an aqueous matrix, oxygen must gain access to the lipid by diffusion into the aqueous phase and passage through the oil-water interface (Nawar, 1996).

The stability of an emulsion can be increased most suitably and effectively by the use of emulsifiers, which unify in the same molecule, a water-repelling "hydrophobic" tail group and a "hydrophilic" head group (Hoffmann, 1989). As these molecules form the interface between the oil and water phases, the surface charge density and thickness of this layer regulates the stability of emulsions (Gunstone, 1996).

As soon as oxygen gains access to the fat, the fatty acid profile and natural antioxidants of the specific emulsified fat will determine the oxidative stability of the emulsion. Oils containing high levels of polyunsaturated fatty acids or less antioxidant are more readily susceptible to oxidation (Gunstone, 1996). The fatty acid profile of mango kernel fat has indicated a high content of stearic (C18:0) and oleic (C18:1) acid, with smaller amounts of palmitic (C16:0), linoleic (C18:2), linolenic (C18:3) and arachidic (C20:0) acids (Baliga & Shitole, 1981, Narasimha Char & Van Pee *et al.*, 1981 & Azeemoddin, 1988).

In this study a stabilised water-in-oil emulsion was made using crude, cold-pressed mango kernel fat (MKF). Oxidative changes in the emulsions were monitored during periodic withdrawals over 240 days by means of the peroxide value (PV), conjugated diene value (CD), and *p*-anisidine value (AV) tests.

Materials and methods

Three individual batches of crude, cold-pressed, mango kernel fat (MKF) were obtained from Specialised Oil cc. (Industria road 2, Industria, Louis Trichardt 0920, South Africa). The clean mango stones were collected from a fruit juice plant (Valley Farms, P.O. Box 163, Levubu 0929, South Africa), followed by manual decortication using a knife. The mango kernels were dried in an electronically controlled oven for 24 hours at 60°C until a moisture content of 12.5% was reached. The dried kernels were then stored at room temperature for three to six days and mechanically pressed at 45°C. The fat was not refined or bleached.

Preparation of emulsion

Soy lecithin (Lecsam H., Bunge Alimentos, Argentina) and distilled monoglyceride (Dimodan SK-A, Danisco Cultor, Denmark) were obtained from Cape Oil, Cape Town and used as emulsifiers. Water-in-oil emulsions were made using a double-jacketed mixing bowl, enclosing a frozen liquid phase. Emulsions were made from each batch using 81% MKF, 18% water, 0.4% monoglyceride and 0.6% lecithin. The oil and water were heated to 50°C, after which the water was added to the oil phase and stirred with an electronic stirrer. After two minutes, the emulsifiers were added and the emulsion was stirred for 30 minutes.

The emulsions were poured into 20 ml clear glass test tubes, filled to the top and closed with Teflon caps to exclude oxygen. Samples were stored for 240 days in dark incubators at 5°C, 15°C, 25°C and 40°C. Enough tubes for every sample were subjected to each storage condition so that no tube that was removed from storage and used for analyses had to be reused.

The crude MKF samples used for comparison were stored at 5, 15, 25 and 40°C, in the dark, in 25 ml Chromacol clear glass bottles. Prior to filling, the oil was liquefied for 10 minutes at approximately 45°C. This was done to ensure a liquid state of the fat during the entire filling time of the bottles before re-solidification of the fat occurred. Bottles were filled to the top and closed with Teflon caps to exclude oxygen.

Analyses

At 40-day intervals, samples from all three batches were removed from the incubators and stored overnight at –80°C. Prior to analyses the emulsions were liquefied at 50°C for approximately 8 minutes whereafter it was centrifuged at 4000 rpm for 6 minutes to separate the oil and water phase. The oil phase was used to determine the oxidative stability of the emulsified MKF by means of the peroxide value, conjugate diene and *p*-anisidine value. The crude MKF samples were liquefied at a temperature of *ca.* 45°C for 10 minutes. This led to an easily obtainable homogenous liquid sample. The samples were opened just before analysis and analyses were done in duplicate for all batches.

The peroxide value was determined according to the American Oil Chemists Society (AOCS) Method Cd 8 53 (1985). Sample sizes were, however, reduced to 0.5g ± 0.0009, using 100 ml instead of 250 ml Erlenmeyer flasks. Volumes were reduced to 10 ml of the acetic acid-chloroform solution, 10 ml of distilled water and a 0.01 M sodium thiosulfate titrant. The conjugated diene value (CD) was determined according to the AOCS Method Ti la-64 (1993). Sample sizes were reduced to 18 ml, diluting to volume with hexane in a 25 ml volumetric flask. The p-anisidine value was determined according to the AOCS Method Cd 18-90 (1992). The absorbances at 233 nm for CD and at 350 nm for AV were measured with a Phillips PU 8700 series, UV/Visible spectrophotometer (Phillips Scientific and Analytical Equipment). The

total oxidation (TOTOX) value was calculated by the formula: TOTOX = 2PV + AV.

Statistical analysis

Graphs were compiled using Statistica version 6. Every point on the graph indicates the average value calculated from two duplicates in three batches (six values). The bar around the average represents the 95% confidence interval for the average. The inspection of the overlapping of the 95% confidence intervals identified significant changes. When no overlapping occurred, significant differences were assumed.

Results and discussion

Peroxide value analysis

After 40 days of storage, the PV of the emulsions stored at 5°C increased from 2.7 meq.kg^{-1} (PV of fresh, crude MKF) to 3.4 meq.kg^{-1}, whilst the PV of samples stored at 15, 25 and 40°C showed a decrease in PV at 40 days (Figure 5.1). This indicated that a higher PV for MKF was possibly reached between 0 and 40 days when stored at 15, 25 and 40°C, as the oxidative process is accelerated at higher temperatures (Narasimhan *et al.*, 2001). On the contrary, it is also possible that emulsification of MKF had a stabilising effect by reducing hydroperoxide production during the emulsification step (Figure 5.2). As hydroperoxides were broken down to form secondary oxidation products, the PV trend line gradually decreased until hydroperoxides were depleted. This decrease was more sudden at 80 days for samples stored at 5°C than for samples stored at 15, 25, and 40°C, indicating the probability of an intermediary step for the 5°C samples between 40 and 80 days.

When comparing MKF (oxygen excluded) stored at 5°C and the mango kernel fat emulsion stored at 5°C (also without oxygen) it is clear that the emulsion reached a higher maximum PV than the crude MKF (Figure 5.2). Apart from that, the trend was very similar for the fat and emulsion except for the more rapid breaking down of hydroperoxides in the emulsion after 40 days, which might be due to the catalysing effect of the moisture encapsulated in the emulsion. It is also clear from Figure 5.3

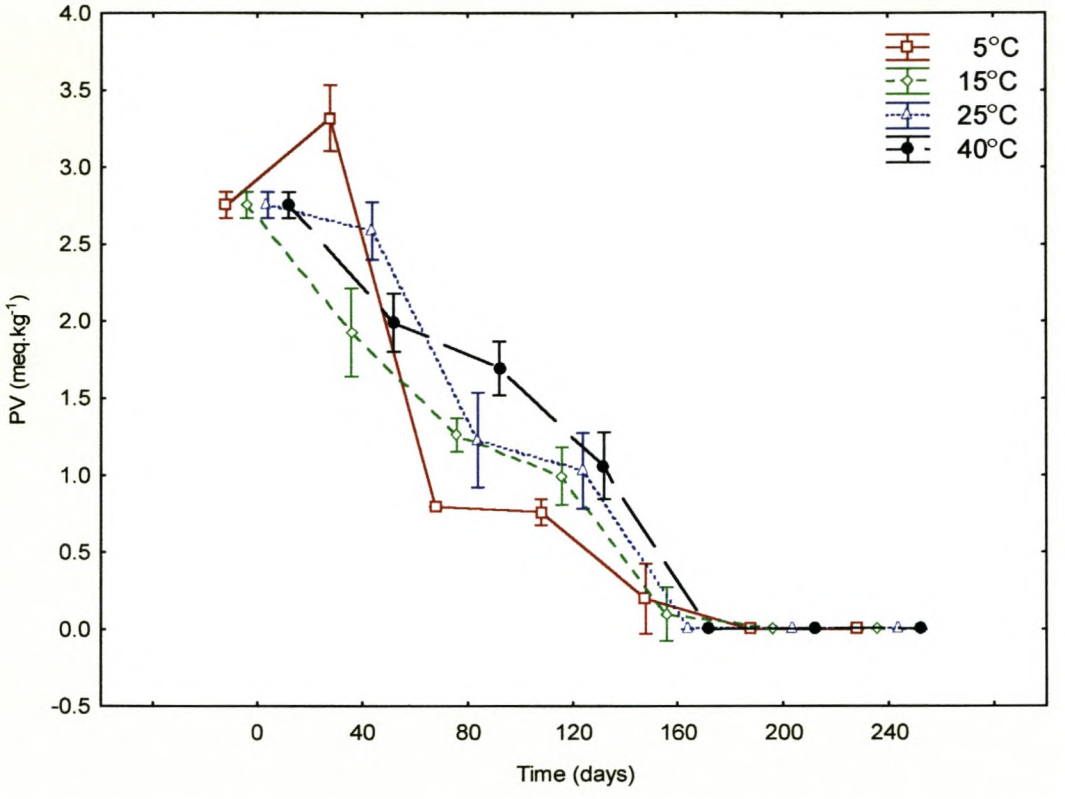


Figure 5.1. Peroxide value of mango kernel fat emulsions, stored at different temperatures for 240 days, with no headspace.

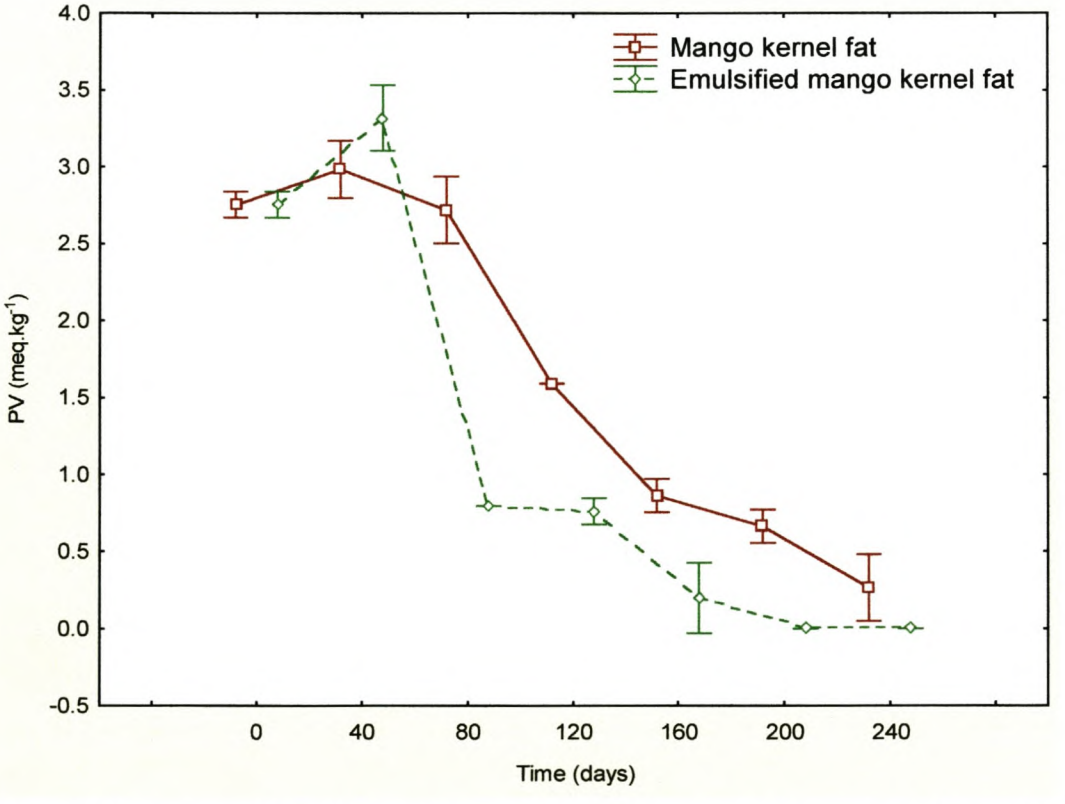


Figure 5.2. Peroxide values of mango kernel fat and mango kernel emulsions, stored at 5°C, for 240 days, with no headspace.

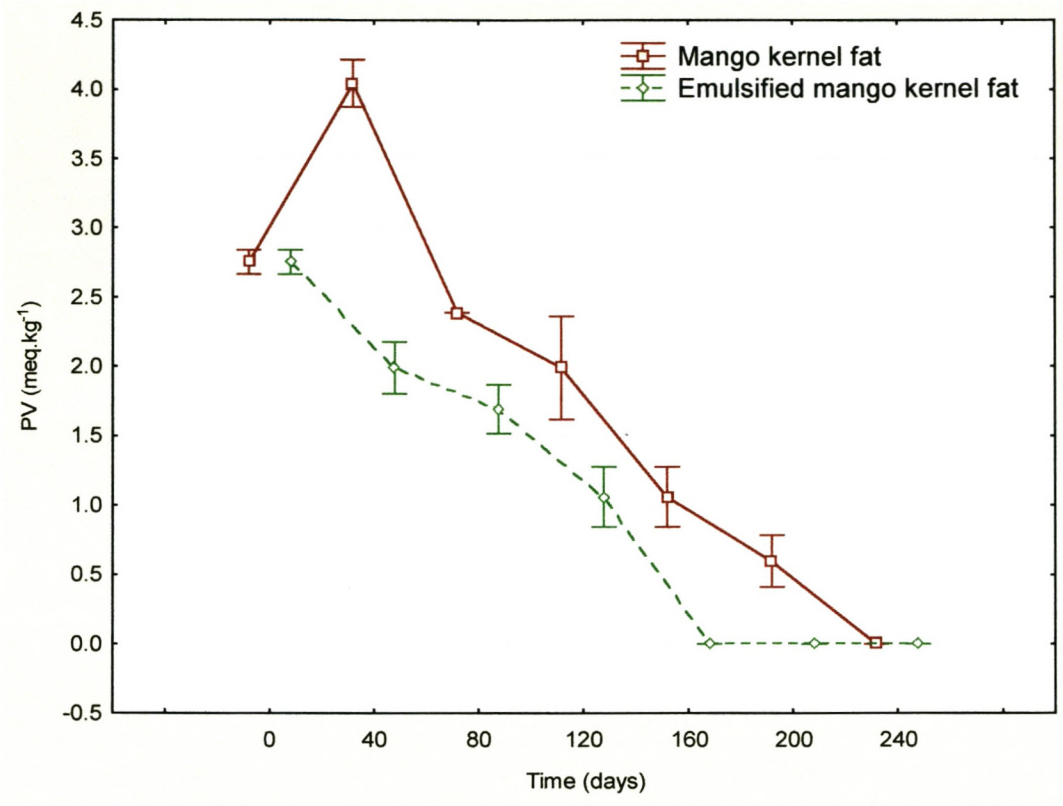


Figure 5.3 Peroxide values of mango kernel fat and mango kernel emulsions, stored for 240 days, with no headspace, at 40°C.

that the rate of hydroperoxide production exceeded that of hydroperoxide breakdown at day 40 for the emulsified sample stored at 40°C, while the crude MKF reaches a maximum PV at day 40. It can therefore be argued that either primary oxidation was completed during the emulsifying stages or a maximum PV was reached between day 0 and day 40.

Conjugated diene value analysis

The conjugated diene (CD) value of the freshly pressed crude mango kernel fat was determined before emulsification at day 0 and had a value of 0.7%. After emulsification and storage (40 – 240 days) of the MKF at different temperatures, the CD value increased to values ranging from 0.14% to 0.18% (Figure 5.4). These CD values were low compared to that of high PUFA oils (up to 6%) (Gunstone, 1996). This was expected as the fatty acid profile of MKF indicates *ca.* 5% polyunsaturated fatty acids. In addition to the low PUFA content, emulsification protects the lipid from oxygen, thus minimising oxidation (Khan & Shahidi, 2000).

Conjugated diene values increased with increasing storage temperature, with samples stored at 5°C having lower maximum values than those stored at 40°C (Figure 5.4). The samples stored at 5°C also had a more stable trend line from day 40 to day 240. Furthermore it can also be seen that samples stored at 5, 15, 25 and 40°C all exceeded the CD values obtained at 40 days. It can be concluded that the conjugated diene formation is not yet completed after 40 days and more of these stable bonds are formed during the later stages of storage. The slightly uneven nature of the trend line after day 40 could be ascribed to the slight biological and chemical differences between samples, especially the differences in unsaturated fatty acid content.

When comparing the emulsified mango kernel fat at 5°C to crude mango kernel fat at 5°C, the CD values were very similar over a period of 240 days (Figure 5.5). Samples stored at 40°C showed slight differences in pattern after 40 days when further formation of conjugated double bonds took place (Figure 5.6).

p-Anisidine value

The *p*-Anisidine value (AV) of freshly pressed mango kernel fat (MKF) was 2.2 $mmol.kg^{-1}$. This value compared well to that of fresh low linoleic soybean oil (AV =

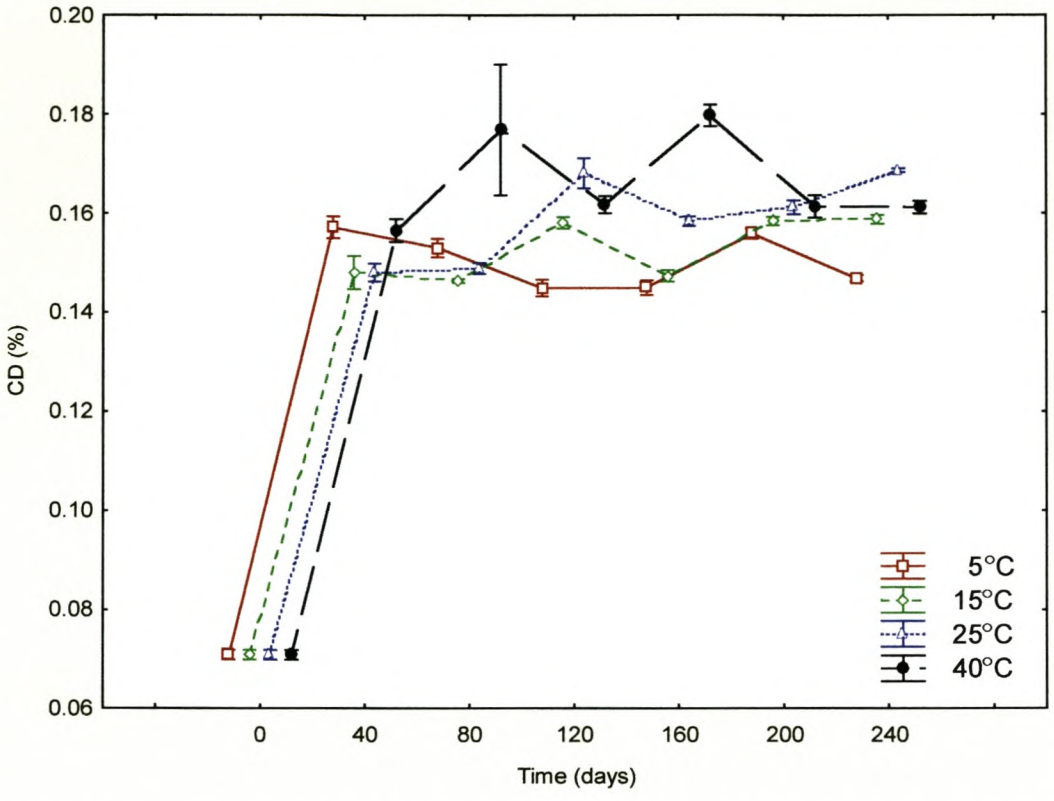


Figure 5.4 Effect of different storage temperatures on conjugated diene values of mango kernel fat emulsions.

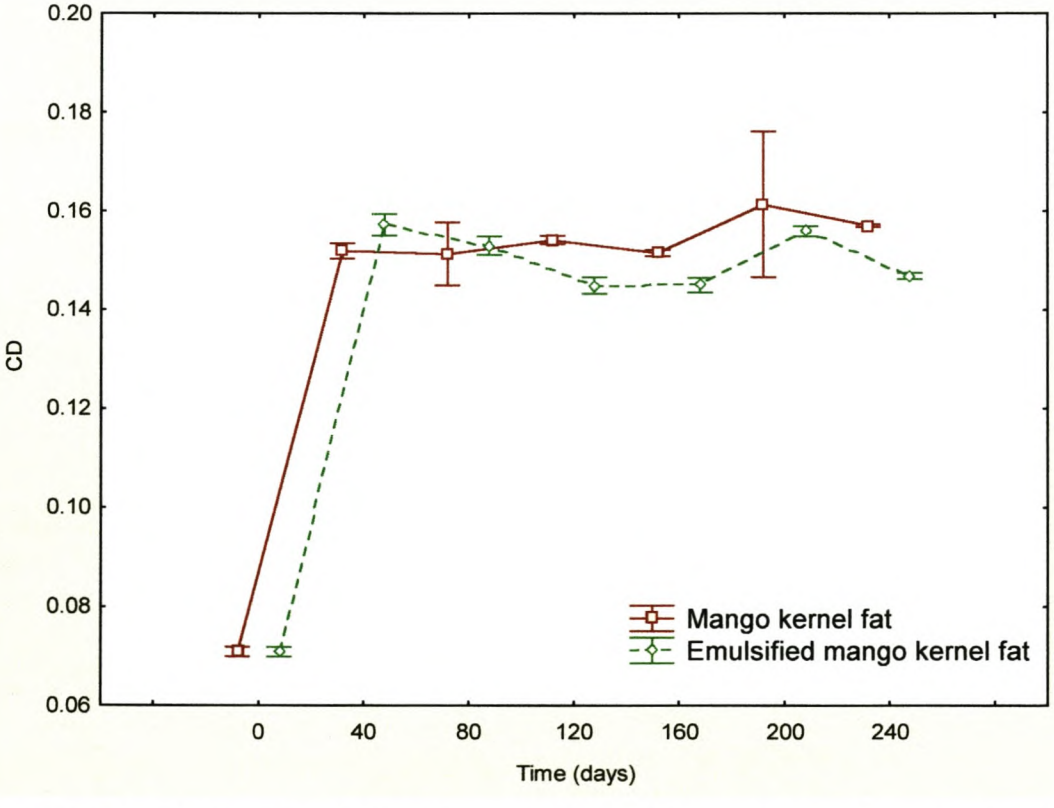


Figure 5.5. Effect of emulsification on conjugated diene values of mango kernel fat during storage at 5°C.

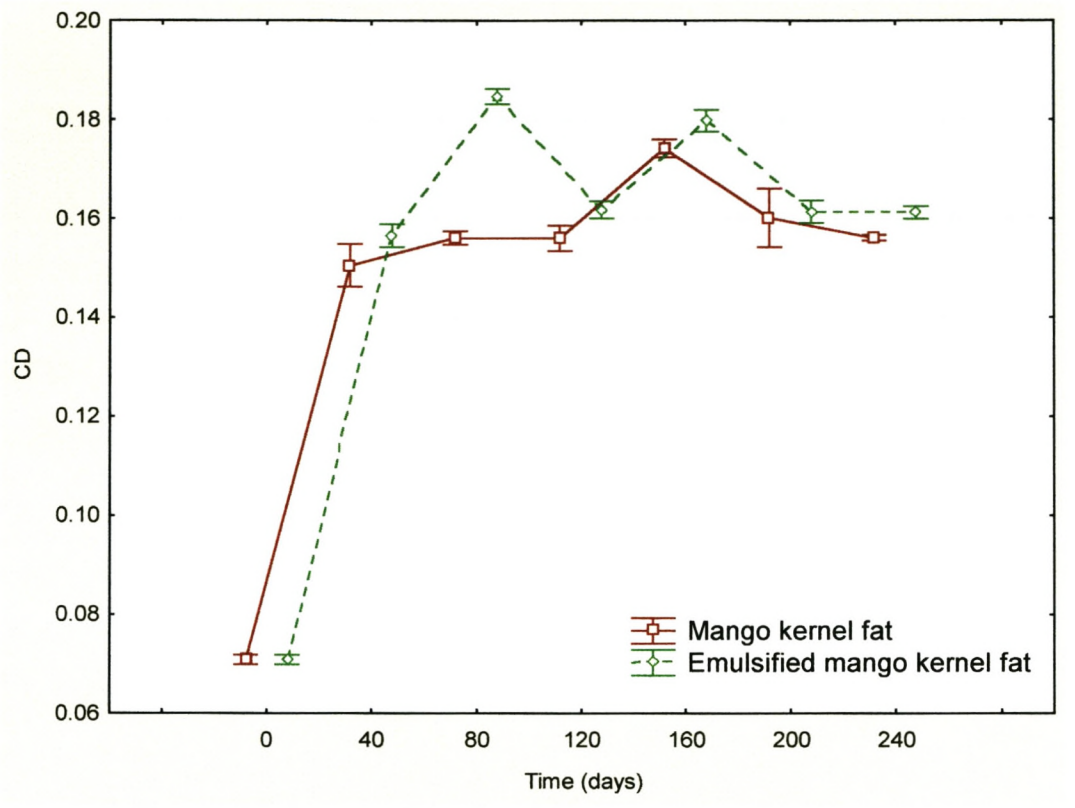


Figure 5.6. Effect of emulsification on conjugated diene values of mango kernel fat during storage at 40°C.

1.52 mmol.kg^{-1}) and sunflower oil (AV = 0.96 mmol.kg^{-1}) (Crapiste *et al.*, 1999; Tompkins & Perkins, 2000). The *p*-anisidine values of emulsified mango kernel fat were low during storage over 240 days (Figure 5.7), ranging mostly between 1.5 and 3 mmol.kg^{-1}. The highest AV of 4.5 mmol.kg^{-1}was reached at 40°C after 40 days. As the AV indicates secondary oxidation products, the higher value at 40 days, correlated well with the primary oxidative results (PV) (Figure 5.1). At 40 days, the hydroperoxides had already been broken down (Figure 5.1) indicating a higher level of secondary products at this stage (Figure 5.7). Even though the *p*-anisidine values for samples stored at 40°C showed variation, the overall AV were very constant indicating a stabilising factor brought about by emulsification.

When comparing the *p*-anisidine values of MKF and emulsified MKF at 15°C over 240 days (Figure 5.8), it is clear that the trend line observed for the emulsion was smoother than that of the crude MKF. The emulsified samples showed a slight increase in secondary products at 40 days and a decrease at 240 dyas, while the crude MKF samples showed a very unstable AV profile throughout days 0 – 240. At a higher temperature however (40°C) the emulsified and crude MKF samples showed exactly the same profile (Figure 5.9) and it can be assumed that emulsification had little influence at a temperature of 40°C.

The overall range of AV was very small when compared to that of heated sunflower oil at 47°C after 60 days (AV=45 mmol.kg^{-1}) (Crapiste *et al.*, 1999). This showed a very small production of the odour causing aldehydes (2,4-dienal and 2-alkenal), and it could be stated that the oxidised mango kernel fat in emulsion did not have a rancid odour. This correlated well with the highest measured peroxide value (PV) of 3.5 meq/kg for emulsified mango kernel fat as in general fat is only considered rancid at a peroxide level of about 10 meq.kg^{-1} (Gunstone, 1996).

TOTOX value

Total oxidation values of emulsified mango kernel fat indicated increased oxidation levels for the samples stored at 5°C after 40 days, while oxidation levels of the samples stored at 15, 25 and 40°C remained constant or decreased (Figure 5.10). These decreases were due to the lower levels of hydroperoxide production after 40 days. When referring back to the peroxide values of these samples (Figure 5.1), it was to be expected that the total oxidation would only show an increase at 40 days for

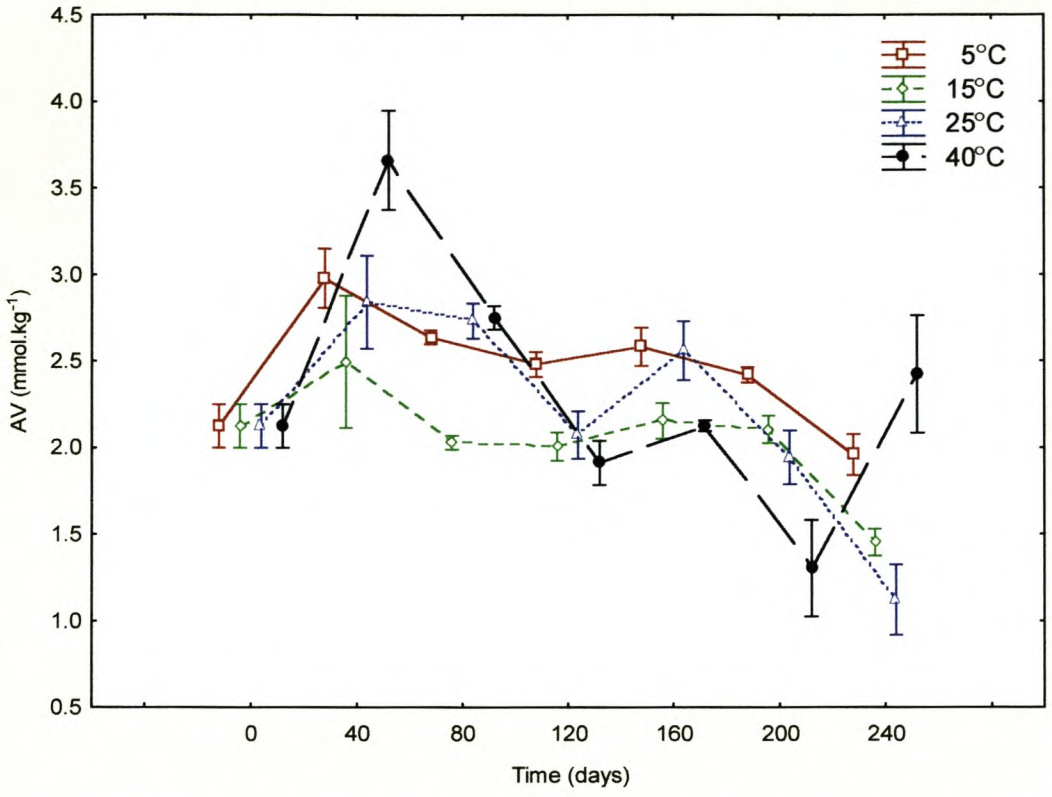


Figure 5.7. The *p*-anisidine value of mango kernel fat emulsions, stored at different temperatures for 240 days, with no headspace.

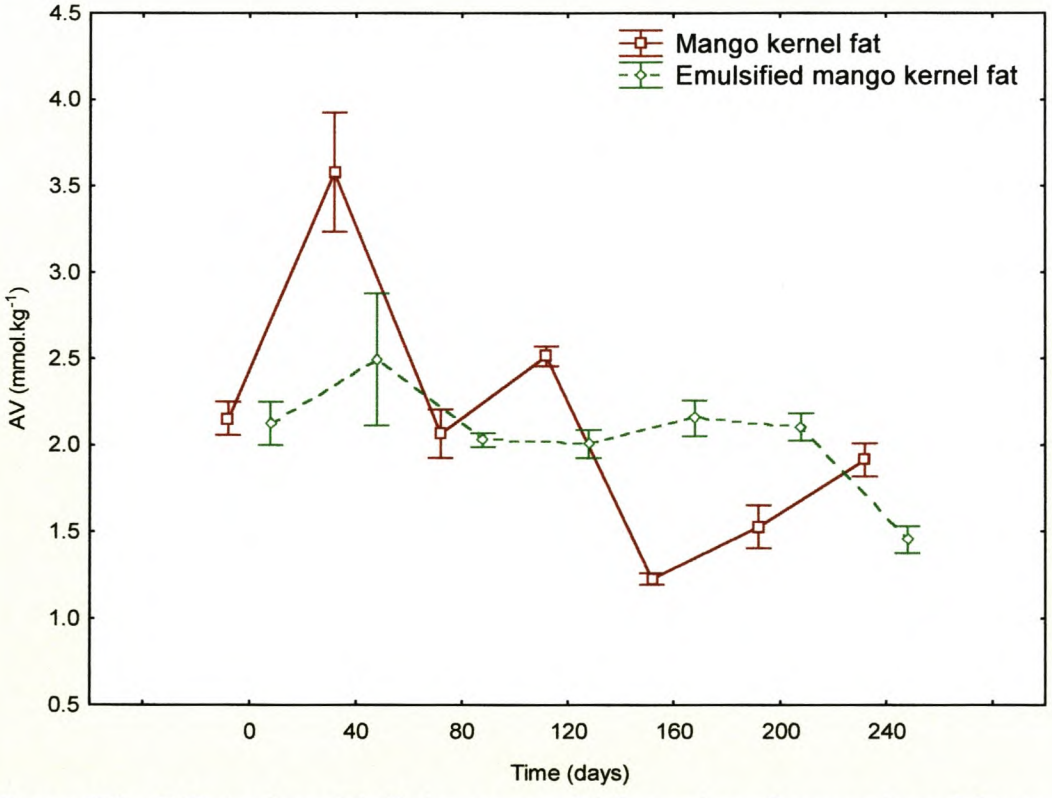


Figure 5.8. Effect of emulsicifation on *p*-anisidine value of mango kernel fat stored without headspace at 15°C.

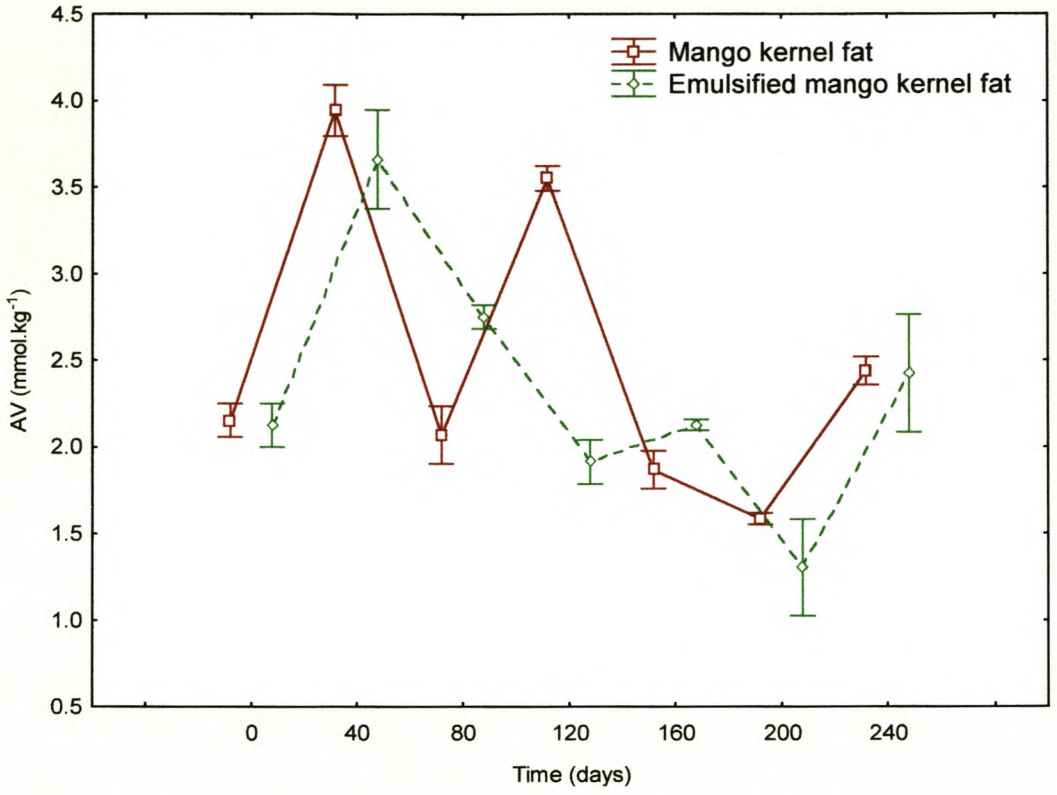


Figure 5.9. Effect of emulsicifation on *p*-anisidine value of mango kernel fat stored without headspace at 40°C.

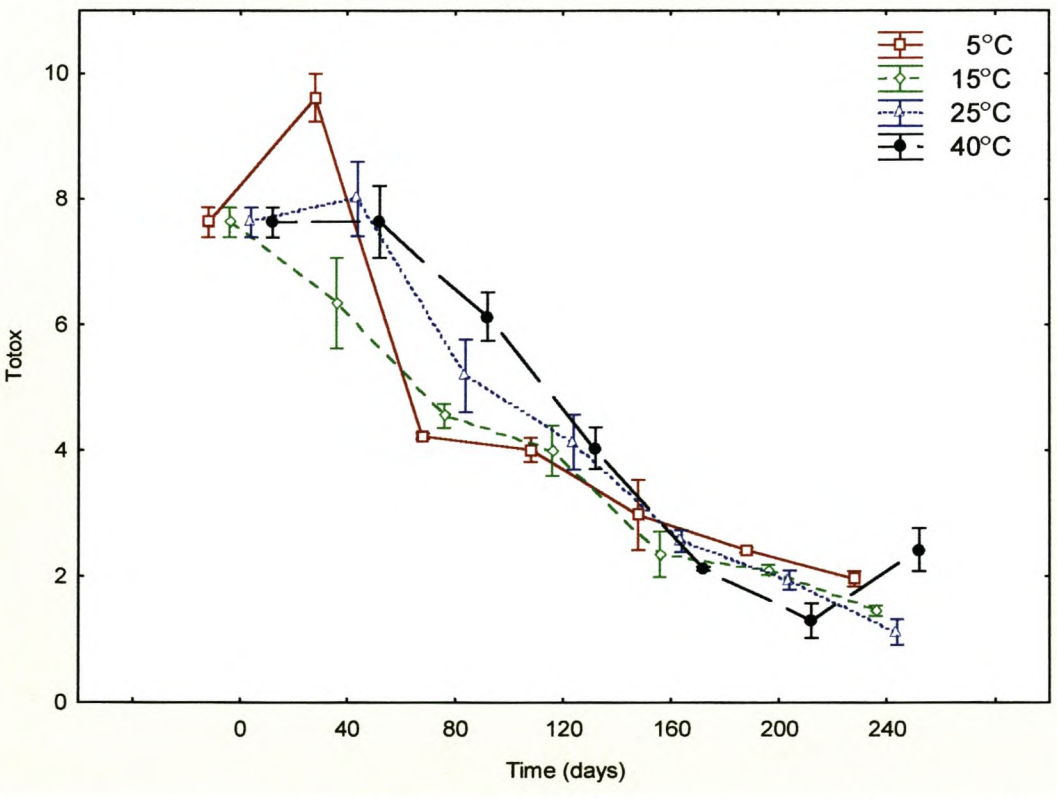


Figure 5.10. Effect of different storage temperatures on total oxidation values of mango kernel fat emulsions.

the samples stored at 5°C as these were the only samples where production of hydroperoxides were noted during the shelf life study. The total oxidation values of the MKF and emulsified MKF had very similar values at 5°C (Figure 5.11). At 25°C, however (Figure 5.12), the stabilising effect of emulsification was more evident.

Conclusion

Emulsification of the mango kernel fat (MKF) seemed to have a stabilising effect against oxidation. Peroxide values (PV) were low, indicating that no rancidity could be detected. Hydroperoxide formation rate was higher than that of hydroperoxide breakdown after 40 days for all temperatures except 5°C and hydroperoxides were broken down to secondary products, which were detected, in limited amounts by the *p*-anisidine value test. The conjugated dienoic test indicated production of more conjugated dienes at higher temperatures, with a very similar trend to that of crude MKF. Both PV and CD methods indicated the inhibition of oxidation due to emulsification of the MKF.

Emulsification increased the fluency of the AV trend line when compared to that of the crude MKF stored without oxygen. This smoothing effect was, however not evident at 40°C. It can therefore be assumed that the stabilising effect of emulsification against the oxidative process was not effective at 40°C. After examination of results of both primary and secondary oxidative stability tests it can be stated that emulsification of crude MKF had a positive influence on the oxidative stability of mango kernel fat.

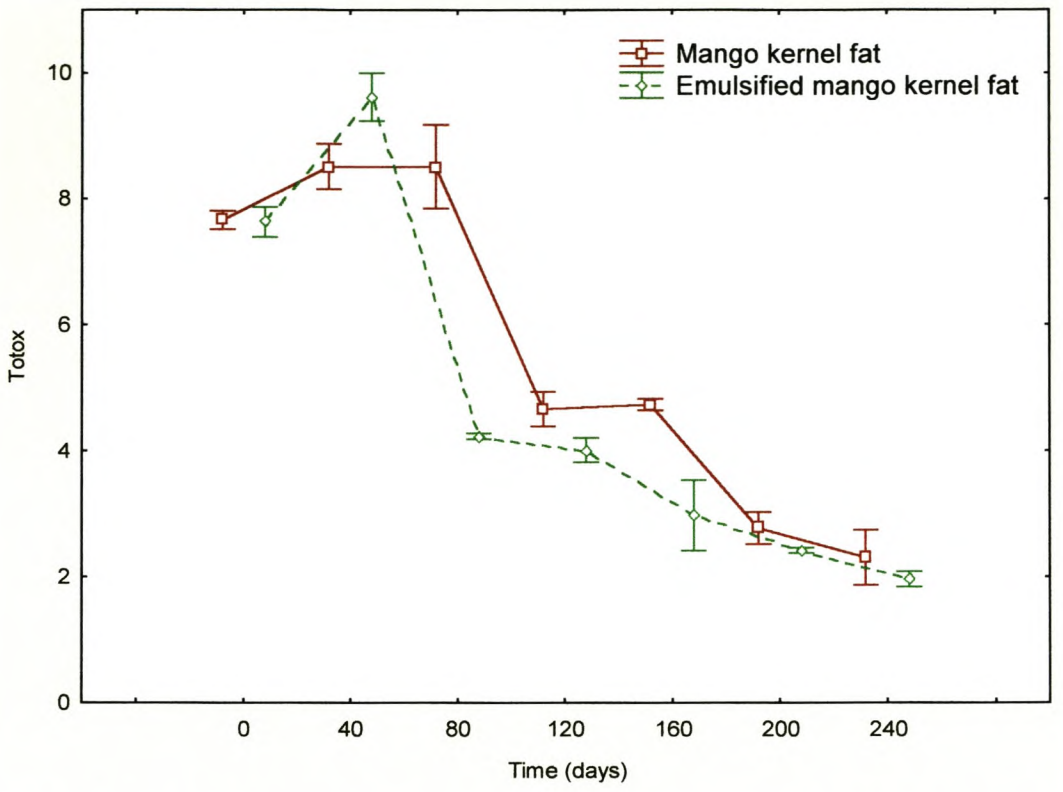


Figure 5.11. Comparison between total oxidation of emulsified mango kernel fat and crude mango kernel fat at 5°C during shelf life study.

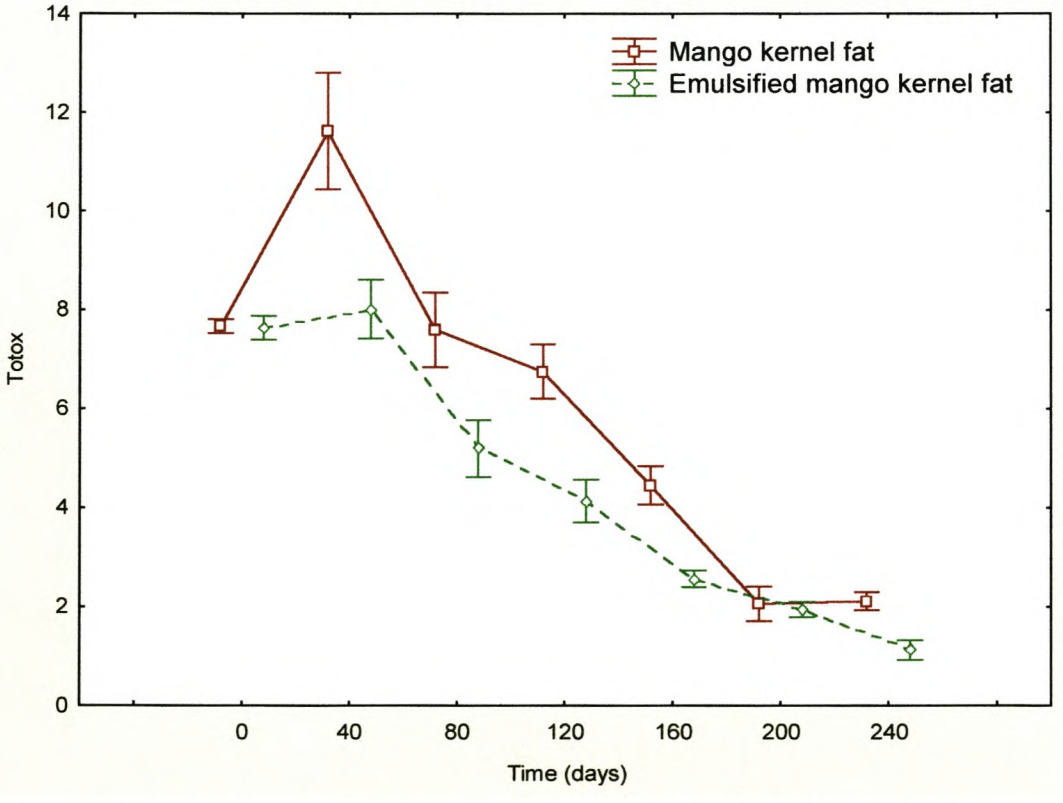


Figure 5.12. Comparison between total oxidation of emulsified mango kernel fat and crude mango kernel fat at 25°C during shelf life study.

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CHAPTER 6

COMPARTIVE STUDY OF VEGETABLE OILS: THE INFLUENCE OF FATTY ACID PROFILE AND STORAGE CONDITIONS ON THE OXIDATIVE STABILITY OF CANOLA OIL, SUNFLOWER OIL, OLIVE OIL AND MANGO KERNEL FAT

Chapter 6

COMPARATIVE STUDY OF VEGETABLE OILS: THE INFLUENCE OF FATTY ACID PROFILE AND STORAGE CONDITIONS ON THE OXIDATIVE STABILITY OF CANOLA OIL, SUNFLOWER OIL OLIVE OIL AND MANGO KERNEL FAT.

Summary

The influence of the fatty acid profiles and storage conditions on the oxidative stability of vegetable oils was studied over a period of 240 days. The oxidative changes occurring in canola, sunflower and olive oil were compared to that of crude, cold-pressed mango kernel fat (MKF). Samples were stored at 25°C, without exposure to oxygen or UV light and monitored every 40 days using the peroxide value (PV), conjugated diene (CD) value and *p*-anisidine value tests. MKF samples were also stored with exposure to UV light and in emulsified form (water-in-oil emulsions). Oxidative changes occurring in these samples were then compared to that of crude MKF, also using PV, CD value and AV tests. Olive and sunflower oils reached a maximum PV of 14.7 meq.kg^{-1} (initial PV = 14.4 meq.kg^{-1}) and 9.8 meq.kg^{-1} (initial PV = 8 meq.kg^{-1}) respectively, while canola oil (initial PV = 3.2 meq.kg^{-1}) and MKF (initial PV = 2.7 meq.kg^{-1}) both reached a maximum PV of 4 meq.kg^{-1}. Although the emulsified MKF never exceeded the PV of the freshly pressed MKF (2.7 meq.kg^{-1}) the samples exposed to UV light reached a maximum PV of 5 meq.kg^{-1}. Initial CD values of 0.07, 0.08, 0.1 and 0.4% increased to 0.17, 0.2, 0.3 and 0.69% for MKF, olive, canola and sunflower oil respectively. Similar maximum CD values were obtained for emulsified MKF and crude MKF, but exposure to UV light, once again catalysed the oxidative process and maximum CD values of 0.21 % were reached. Maximum *p*-anisidine values of 10, 5, 3.2 and 3.5 mmol.kg^{-1} were reached for sunflower, olive and canola oil and MKF. These values displayed an average increase of one unit from the original *p*-anisidine values obtained. Emulsification of the MKF once again produced a lower AV, while samples exposed to light had an AV of 5.3 mmol.kg^{-1}. Values obtained for the PV, CD and AV tests correlated well with the linoleic acid (C18:2) contents of the vegetable oils as obtained from literature. The total oxidation values (TOTOX) obtained were 34 units for olive oil, 29 units for sunflower, 10 units for canola oil and 12 units for MKF.

Introduction

Lipid oxidation is an important reaction occurring between unsaturated lipids and atmospheric oxygen (Nawar, 1996). The oxidation process is accelerated by metals, light, heat and several initiators (Gunstone, 1996). It can be inhibited by antioxidants. Lipid oxidation occurs under enzymatic and non-enzymatic conditions and the latter can operate through autoxidation or photo-oxidation (Gunstone, 1996).

From the view of food oxidation, the important lipids are those containing unsaturated fatty acids, particularly oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) (White, 1995). The susceptibility and rate of oxidation of these fatty acids increase in relation to their degree of unsaturation (Gunstone, 1996). The greater the number of double bonds, the greater the probability that the fatty acid will react with oxygen to generate undesirable flavours in the product (Marsili, 1993). In addition, oxidative reactions can decrease the nutritional quality of food and certain oxidation products are potentially toxic (Nawar, 1996).

The primary oxidation products are allylic hydroperoxides, which subsequently break down to form secondary oxidation products (alcohols, ketones and aldehydes), leading to off-flavours (Moh *et al.*, 1999). Primary oxidative products can be monitored by peroxide value (PV), weight gain, loss of unsaturated fatty acids and conjugated diene value (CD) (Crapiste *et al.*, 1999). Secondary changes are generally measured by *p*-anisidine value (AV), thiobarbituric acid test (TBA) and chromatographic techniques. The PV and AV may be combined to form the total oxidation or TOTOX value (Hoffmann, 1989).

In a study on crude and refined sunflower oils, Crapiste *et al.* (1999) concluded that the rate of oxidation was strongly dependent on oxygen concentration and temperature. They also found that the relative rate of autoxidation increased with the polyunsaturated level, particularly that of linoleic acid. Chu & Kung (1998) noted the increased level of oxidation due to polyunsaturation when monitoring the oxidative stability of canola and olive oil, both which have high levels of polyunsaturated fatty acids (PUFA). Linoleic acid (C18:2) concentrations in sunflower (67.9%), canola (21.7%), and olive (10.7%) oils are high, thus rendering these oils prone to oxidation. Satue *et al.* (1995) oxidised olive oil for 15 days at 60°C in the dark and obtained peroxide values ranging from 11 – 32.5 meq.kg^{-1}. For sunflower oil stored at 67°C, AV remained practically constant at the earlier stages of oxidation, but then increased

sharply following the decomposition of peroxides, reaching a value of 50 after 60 days of storage at 47°C (Crapiste *et al.*, 1999).

Polyunsaturated fatty acids are becoming more popular as the health trend is moving away from the use of saturated fats to oils containing high levels of monounsaturated and polyunsaturated fatty acids (Marsili, 1993). Although this may mean a healthier product for the consumer, it can also cause more problems for food processors because of the increased potential for oxidation and off-flavour development (Gunstone, 1996).

In developing countries, where the shortage of edible oils is a cruel reality, the PUFA content is a luxury that cannot be afforded (Narasimha Char & Azeemoddin, 1988). In view of the shortage of edible oils in developing countries, nutritional and toxicological evaluations have been carried out on some oils from novel sources such as agricultural waste to determine whether they might be safe for human consumption (Polasa & Rukmini, 1987). One of these agricultural waste products is the stone (or seed) derived from the mango fruit (Puravankara *et al.*, 2000). The kernel inside this stone contains fat that is characterised by large amounts of saturated fatty acids namely palmitic (C16:0; 6–16%) and stearic (C18:0; 24-49%) acids; thereby making it more stable against oxidation (Kostermans & Bompard, 1993).

The aim of the study was to compare the oxidative stability of MKF, sunflower oil, canola oil and olive oil during storage in the dark, for 240 days, at 25°C. The influence of emulsification and UV light was also investigated. Primary and secondary oxidation was determined by means of the PV, CD and AV tests.

Materials and methods

Sunflower, canola and olive oil

Three batches of canola, sunflower and olive oil were purchased from three different supermarkets in the Western Cape, South Africa. Care was taken to avoid duplication of samples and this was done by tracking *sell by* dates and batch codes, if available, from the packaging.

Mango kernel fat (MKF)

Three individual batches crude, cold-pressed, mango kernel fat (MKF) were obtained from Specialised Oil cc. (Industria road 2, Industria, Louis Trichardt 0920, South

Africa). The clean mango stones were collected from a fruit juice plant (Valley Farms, P.O. Box 163, Levubu 0929, South Africa), followed by manual decortication using a knife. The mango kernels were dried in an electronically controlled oven for 24 hours at 60°C until a moisture content of 12.5% was reached. The dried kernels were then stored at room temperature for three to six days and mechanically pressed at 45°C. The fat was not refined or bleached.

Emulsification

Soy lecithin (Lecsam H., Bunge Alimentos, Argentina) and distilled monoglyceride (Dimodan SK-A, Danisco Cultor, Denmark) were obtained from Cape Oil, Cape Town and used as emulsifiers. Mango kernel fat water-in-oil emulsions were made using a double-jacketed mixing bowl, enclosing a frozen liquid phase. Emulsions were made from each batch using 81% MKF, 18% water, 0.4% monogliceryde and 0.6% lecithin. The oil and water were heated to 50°C, after which the water was added to the oil phase and stirred with an electronic stirrer. After two minutes, the emulsifiers were added and the emulsion was stirred for 30 minutes.

The emulsions were poured into 20 ml clear glass test tubes, filled to the top and closed with Teflon caps to exclude oxygen. Samples were stored for 240 days in dark incubators at 5°C, 15°C, 25°C and 40°C. Enough tubes for every sample were subjected to each storage condition so that no tube that was removed from storage and used for analyses had to be reused.

Storage

The MKF, sunflower, canola and olive oils were stored in 25 ml chromacol clear glass bottles (Figure 6.1). Prior to filling, the MKF was liquefied for 10 minutes at approximately 45°C. Because of the high PUFA content of the rest of the samples, no heating was required. Samples were filled to the top and closed with a Teflon cap to ensure total oxygen exclusion. Samples were stored in the dark for 240 days at 25°C. Duplicates of these MKF samples were also stored at 25°C, but exposed to UV light. Enough vials for every oil sample were subjected to each storage condition so that no vial had to be reused once removed from storage and used for analyses.

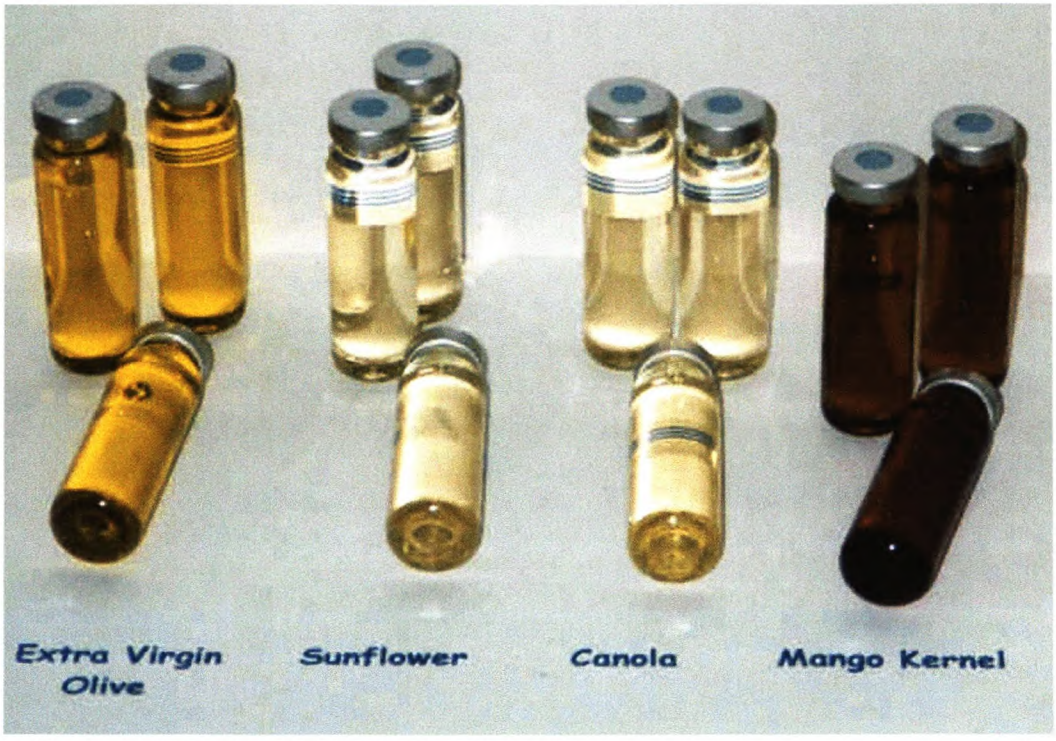

Figure 6.1. Appearance of olive, sunflower and canola oils and mango kernel fat stored in 25ml chromacol glass vials with exclusion of oxygen.

Analytical

At 40-day intervals, samples from all three MKF batches were liquefied at a temperature of *ca.* 50°C for 10 minutes. This was necessary as MKF was still solidified at 25°C. This led to an easily obtainable, homogenous liquid sample. The samples were shaken lightly and opened just before analysis. Analyses were done in duplicate for all batches.

The peroxide value was determined according to the American Oil Chemists Society (AOCS) Method Cd 8 53 (1985). However, sample sizes were reduced to 0.5g ± 0.0009, using 100 ml instead of 250 ml Erlenmeyer flasks. Volumes were reduced to 10 ml of the acetic acid-chloroform solution, 10 ml of distilled water and a 0.01 N sodium thiosulfate titrant. The conjugated diene value (CD) was determined according to the AOCS Method Ti la-64 (1993). Sample sizes were reduced to 18 ml, diluting to volume with hexane in a 25 ml volumetric flask. The *p*-anisidine value was determined according to the AOCS Method Cd 18-90 (1992). The absorbances at 233 nm for CD and at 350 nm for AV were measured with a Phillips PU 8700 series, UV/Visible spectrophotometer (Phillips Scientific and Analytical Equipment). The total oxidation (TOTOX) value was calculated by the formula: TOTOX = 2PV + AV.

Statistical Analysis

Graphs were compiled using Statistica version 6. Every point on the graph indicates the average value calculated from two duplicates in three batches (six values). The bar around the average represents the 95% confidence interval for the average. The inspection of the overlapping of the 95% confidence intervals identified significant changes. When no overlapping occurred, significant differences were assumed.

Results and discussion

Peroxide value

The peroxide values of the sunflower and olive oils were generally higher than that of the MKF (Figure 6.2). This was expected, as the fatty acid profiles show a high percentage of PUFA. The canola oil samples show stability, with peroxide values ranging from 0 to 4 meq.kg^{-1}. This stability can be ascribed to higher amounts of natural antioxidants present in the oil (Satue *et al.*, 1995). It may also indicate that the

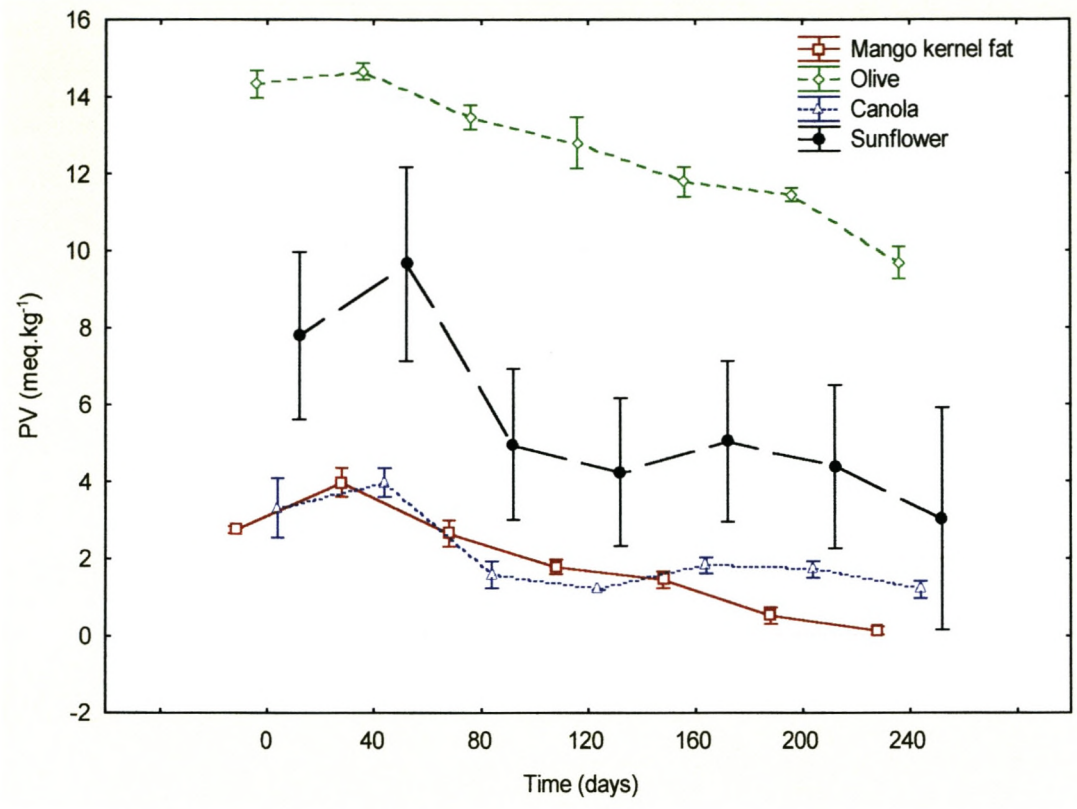

Figure 6.2. Changes in peroxide values of mango kernel fat, olive, canola and sunflower oil at 25°C over a 240-day storage period.

samples obtained from the supermarket were much less oxidised than the sunflower or olive oil samples. The olive oil had a maximum PV of 14.7 meq.kg^{-1}, indicating rancidity. The initial PV of the oil was already high, showing that the oil was in an advanced stage of oxidative deterioration before the samples were purchased. This is surprising, as the sunflower oil was expected to have the highest PV due to the fact that the linoleic acid content of sunflower oil is the highest of the oils tested. Although the sunflower oil reached a lower maximum PV, it is also near 10 meq.kg^{-1}, indicating a suggestion of rancidity. The MKF, on the other hand, shows excellent stability, not exceeding a PV of 4 meq.kg^{-1}. This was expected, as the linoleic acid concentration of MKF did not exceed 6%. According to the generally expected value of freshly pressed vegetable oil (PV=1 meq.kg^{-1}) (Gunstone, 1996), the canola and MKF were the only oils that were not in an advanced stage of primary oxidation at the commencement of the shelf life study.

When the peroxide values of MKF stored at 25°C under different conditions (emulsification & UV light) were compared, the stabilising effect of emulsification could be observed. At the same time, the destabilising effect of light on oxidative stability could be detected when looking at the higher PV of the MKF sample stored under UV light. The PV of the samples stored under UV light increased to 5 meq.kg^{-1}, while that of the MKF samples stored in the dark did not exceed 4 meq.kg^{-1}. The sudden lowering in PV of the sample exposed to UV light at day 80 can also be ascribed to the increased oxidative deterioration. The increased rate of hydroperoxide production reached a maximum at 40 days, whereafter the UV exposure catalysed the breakdown of hydroperoxides, causing a rapid decrease in the peroxide values. The maximum PV of the emulsified mango kernel fat never exceeded 3 meq.kg^{-1}, indicating oxidative stability. The breakdown of hydroperoxides was also more gradual, showing a stable environment with no factors increasing the oxidation rate. Low PV for the MKF emulsion could also be attributed to the fact that oxidation had already started during the emulsification phase. The MKF sample stored in the dark was a good example of the normal process of oxidation without any inhibitory or catalysing effects on the tempo of oxidation. The graph depicted in Figure 6.3 is therefore a good example of the influence of light and emulsification on the oxidative stability of MKF.

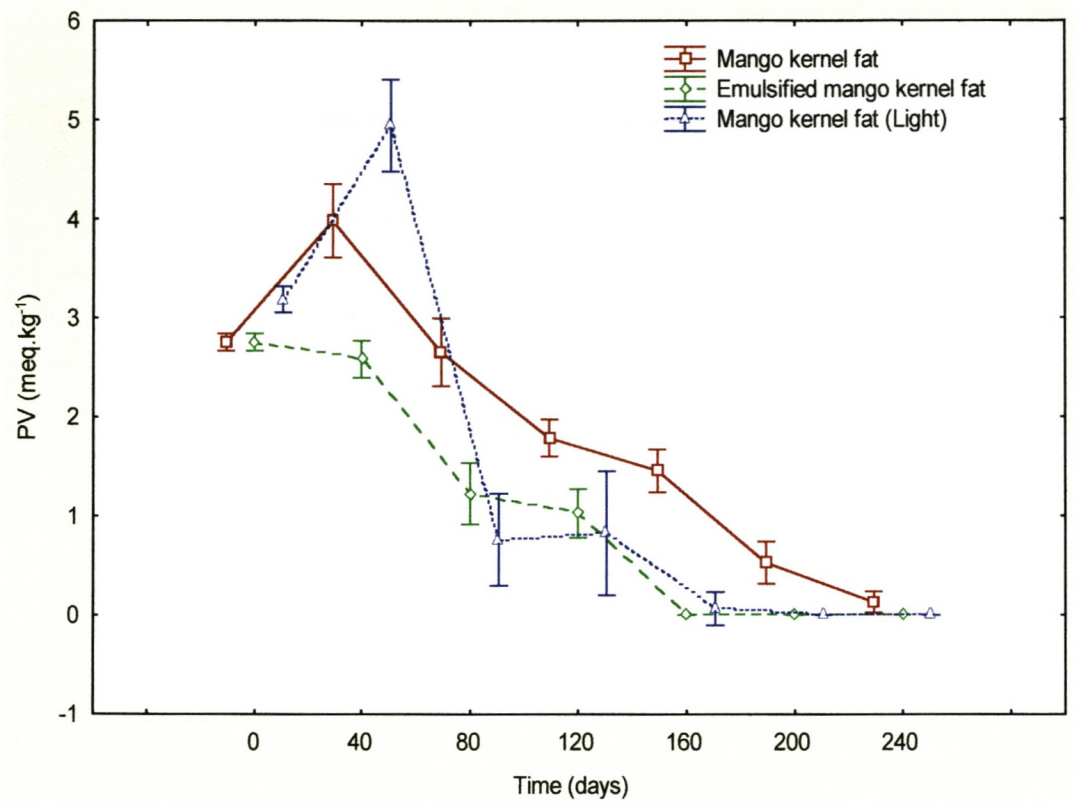

Figure 6.3. Changes in peroxide values of mango kernel fat under different conditions at 25°C over a 240-day storage period.

Conjugated diene values

Conjugated diene values (CD) of the oils decreased in order of diminishing linoleic acid content. As expected, the sunflower oil samples reached the highest CD (0.7%) (Figure 6.4); as the fatty acid profile of sunflower oil consists of *ca.* 70% linoleic acid (Chu & Kung, 1998). This implies that more conjugated dienoic bonds can be formed within the oil during oxidative deterioration. Canola oil, with a linoleic acid content of *ca.* 22%, showed a lower CD of 0.3%. Olive oil and MKF, with a linoleic acid content each of *ca.* 11% and *ca.* 5% showed similar values of 0.1% – 0.2%, with olive oil constantly being slightly higher. These values were an excellent example of the double bond shifts occurring during oxidative deterioration. Both canola and olive oil demonstrated an increase in CD values (from 0 – 40 days), whereafter the values remained constant with the conjugated dienes in the most stable form. The same increase could be observed for sunflower oil, although a bit of destabilisation occurred towards the end of the study. This destabilisation could be neglected when taking the 95% confidence intervals into account. MKF also demonstrated an increase in conjugated dienes up to 40 days, whereafter the stable conjugated dienes were formed and no further changes observed.

When comparing the CD of MKF at different conditions (Figure 6.5), it can be observed that MKF and emulsified MKF show a very similar trend, with the CD value rising at 40 days and ranging between 0.15% and 0.17% for the rest of the study. The MKF samples exposed to UV light showed a different trend, by increasing from 0.01% to 0.11% during the first 40 days and then increasing to 0.17% after 80 days and to 0.21% at 240 days. The influence of UV light was clearly exhibited, as the CD values of the MKF samples exposed to UV light were significantly higher than those of the MKF and emulsified MKF. Since there were so few double bonds present in the fatty acid profile of the fat, the low CD values (0.18%) of MKF during the shelf life study were not surprising.

p-Anisidine value analysis

The highest *p*-anisidine values (AV) were observed for sunflower oil (Figure 6.6), reaching values of up to 10. This was to be expected, as the breakdown products of linoleic and linolenic acids are measured as 2,4-dienal and 2-alkenal. The low PV of canola oil (Figure 6.1) does not really allow a high AV, as the amount of breakdown

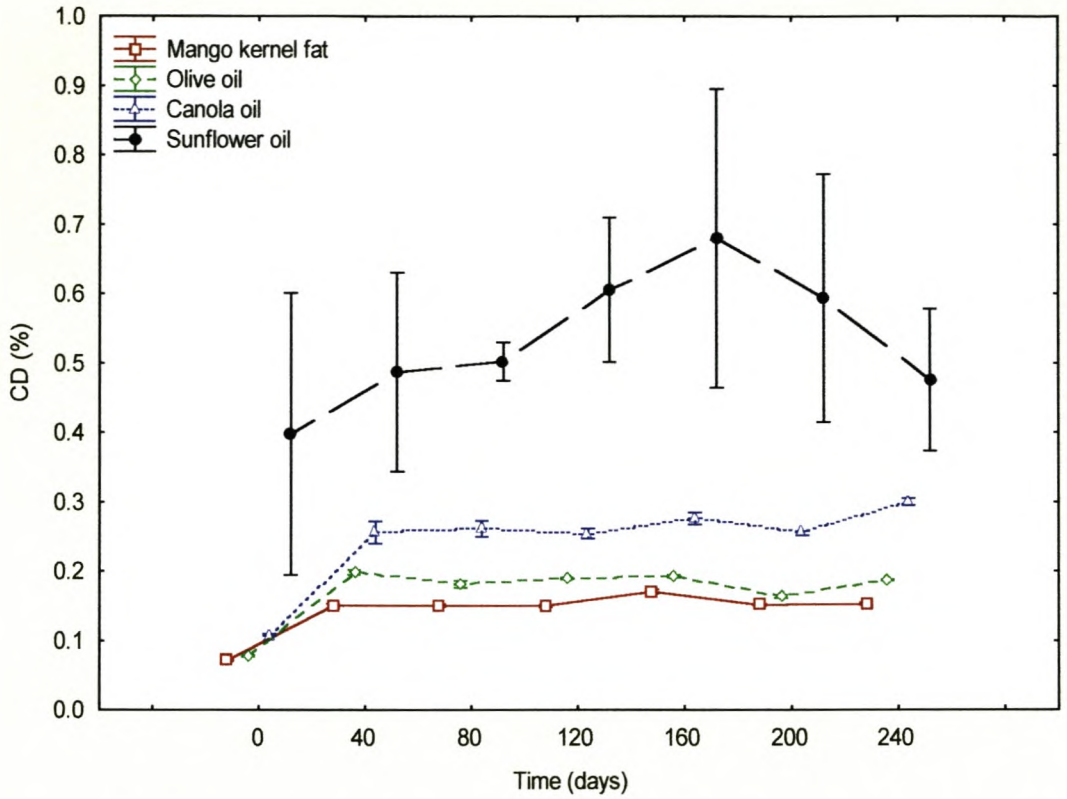

Figure 6.4. Changes in conjugated diene values of mango kernel fat, olive, canola and sunflower oil at 25°C over a 240-day storage period.

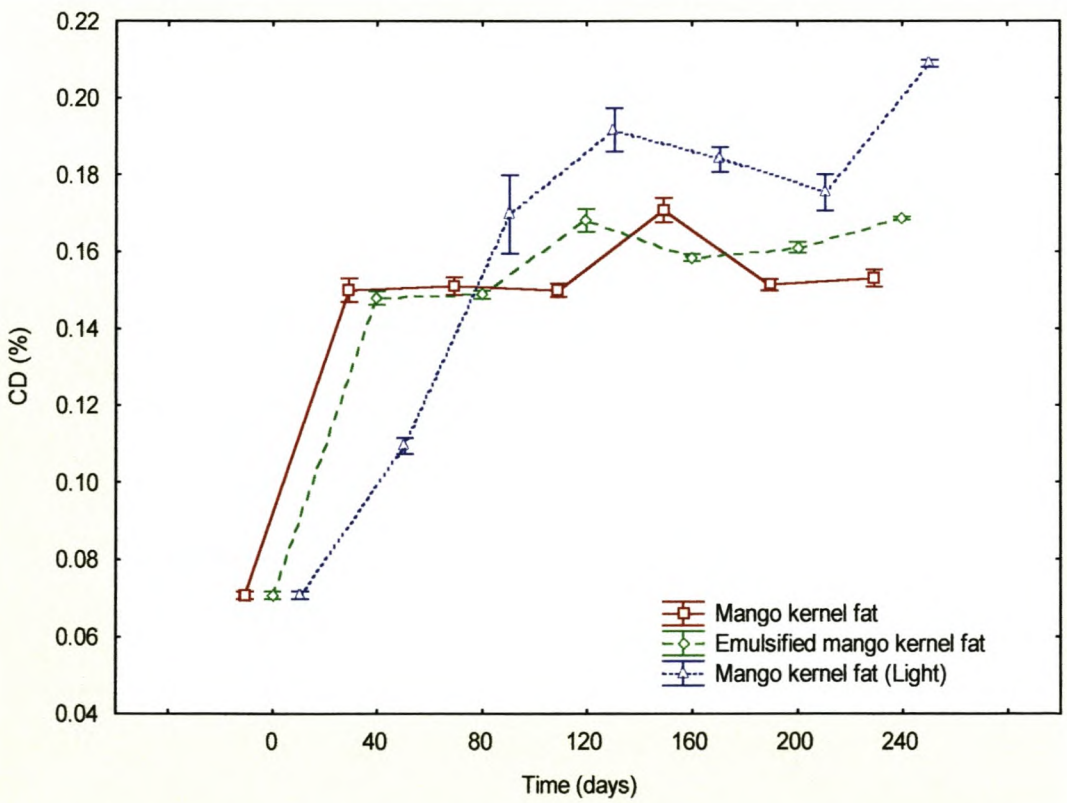

Figure 6.5. Changes in conjugated diene values of mango kernel fat under different conditions at 25°C over a 240-day storage period.

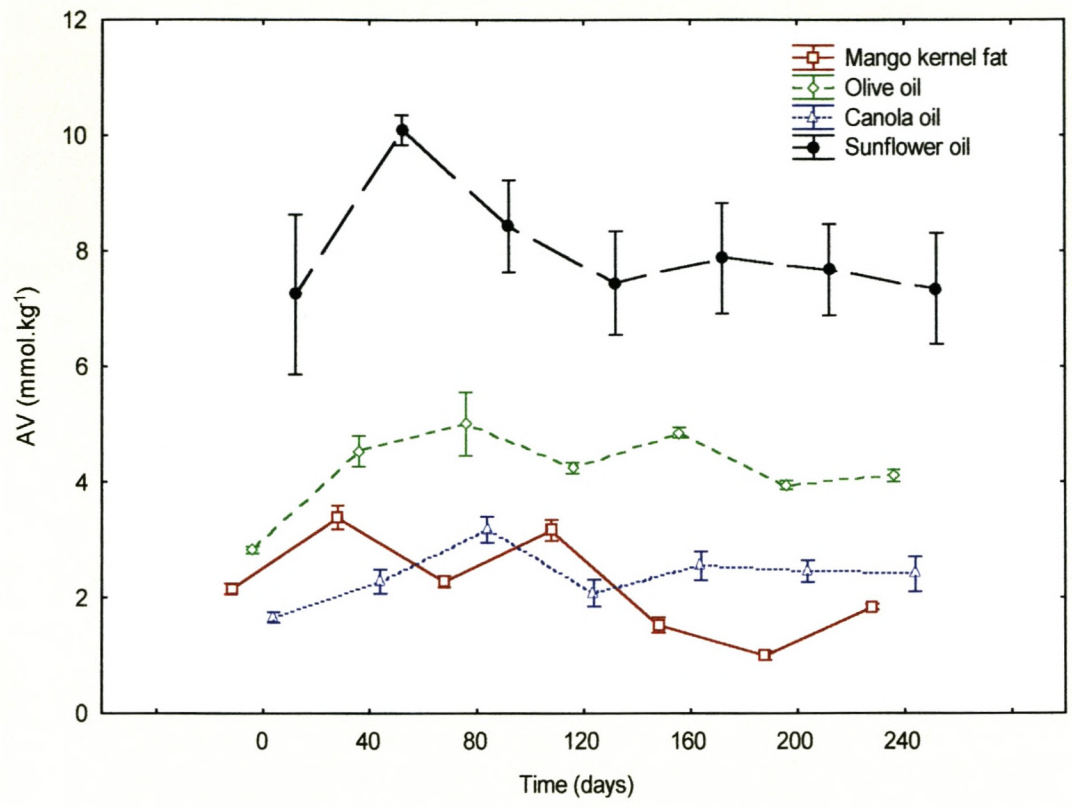

Figure 6.6. Changes in *p*-anisidine values of mango kernel fat, olive, canola and sunflower oil at 25°C over a 240-day storage period.

products than can be formed are limited. The AV curve was smooth, correlating well with the PV curve, which also did not decline drastically. It was expected that the AV would rise with longer storage, as more 2,4-dienal and 2-alkenal were produced. The natural antioxidants in the olive oil (tocopherol & polyphenols) could also contribute to the delayed production of secondary products (Satue, 1995). The low *p*-anisidine values for MKF confirmed the saturated nature of the fat in comparison with the higher AV values of the high PUFA oils. The fatty acid profile of MKF consists mainly of oleic acid (C18:1), which breaks down to form nonanal, which could not be correlated with the AV (Tompkins & Perkins, 1999). The minor variation observed, could be ascribed to the small amount of PUFA oxidised and occurring in differing concentrations in the small volumes analysed as well as biological and chemical variation due to the fact that the fat was unrefined.

When comparing MKF samples stored under different conditions, the stabilising effect of emulsification was once again visible (Figure 6.7). The curve of the emulsified mango kernel fat was much less erratic than that of the MKF or MKF exposed to UV light. The MKF sample exposed to UV light showed a drastic increase in AV at day 40, which correlated well with the PV (Figure 6.3) and CD value (Figure 6.5) curves. This might be due to conditions causing a drastic increase in oxidation products at this stage in the oxidative deterioration process. The increase in both primary and secondary oxidation products was detected at the same time. This cannot be due to experimental error, as three sets of duplicates were done for each experiment and different methods and chemicals were used on different days to carry out the experiments.

TOTOX values

As expected, the total oxidation profiles of the oils showed the same pattern as that of the peroxide values (Figure 6.8). The sunflower and olive oil values were closer together, though, due to the high *p*-anisidine values of the sunflower oil. Compared to MKF and canola oil, the values for olive and sunflower oil were very high. It could therefore be assumed that oils tested were oxidised in the following decreasing order: Olive, sunflower, canola oil and MKF. The total oxidation trend lines for MKF at 25°C for 240 days clearly indicate that light had a catalysing effect on oxidative deterioration (Figure 6.9), as can be seen by the elevated value at day 40.

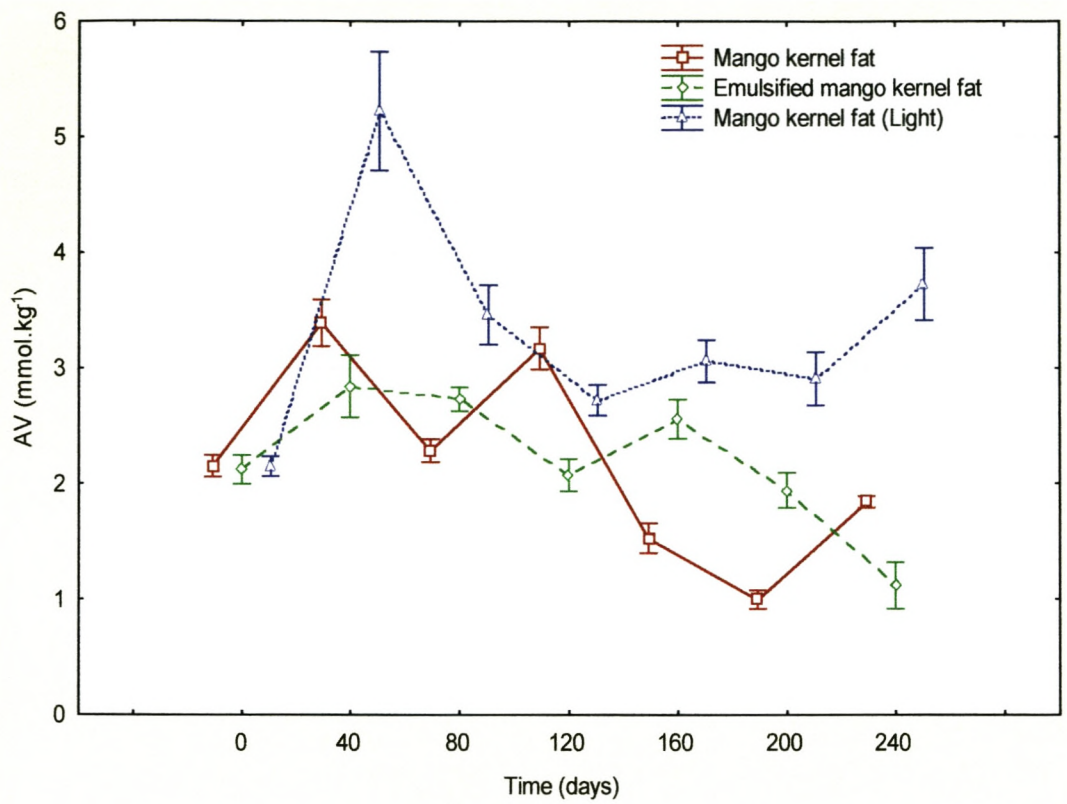

Figure 6.7. Changes in *p*-anisidine values of mango kernel fat under different conditions at 25°C over a 240-day storage period.

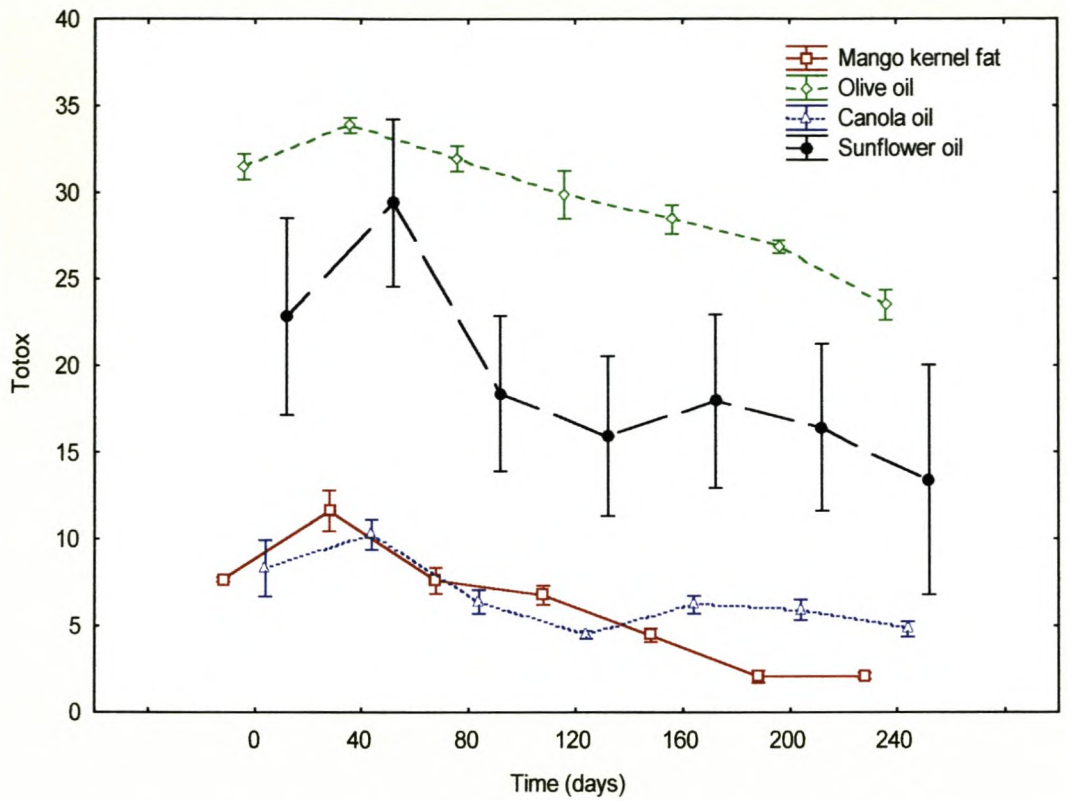

Figure 6.8. Changes in total oxidation of mango kernel fat, olive, canola and sunflower oil at 25°C over a 240-day storage period.

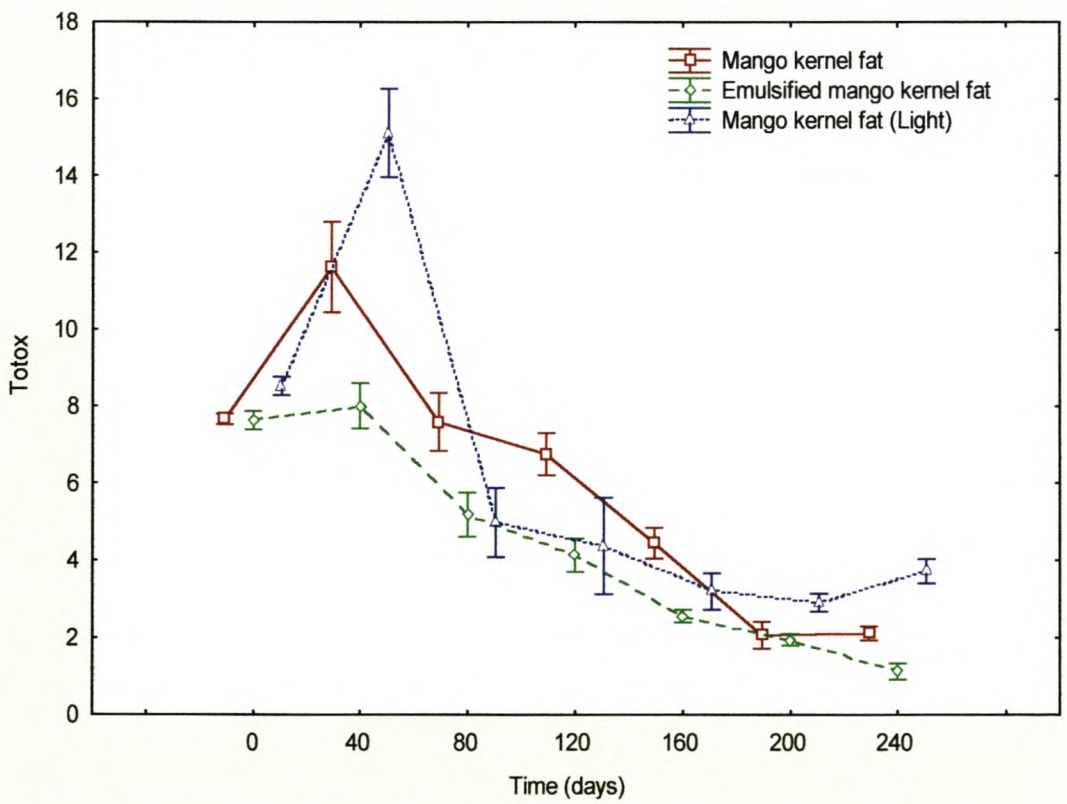

Figure 6.9. Changes in total oxidation of mango kernel fat under different conditions at 25°C over a 240-day storage period.

Consequently, the emulsification of the mango kernel fat had a stabilising effect on oxidative deterioration. Total oxidation does not reach a high maximum for the emulsified or the crude MKF. The samples exposed to light, on the other hand, rise significantly higher, pointing to a more noteworthy state of oxidation. Although the TOTOX value is only an indication of oxidative deterioration, Figure 6.9 is an indication of the influence of storage conditions on the oxidative stability of mango kernel fat.

Conclusion

The oxidative stability tests proved the negative influence of polyunsaturated fatty acids (PUFA) on oxidative stability. Confirmation of the oxidative stability of crude mango kernel fat was also obtained from the results of the PV, CD and AV tests. The PV indicated that oils with high amounts of PUFA produced more hydroperoxides at 25°C than those with a minimal amount of PUFA. It was also concluded that the initial oxidative state of the oil before commencement of the shelf life study had a significant influence on the oxidative performance of the oil. The presence of natural antioxidants in the oils cannot be excluded, however. The influence of different storage conditions was also observed for MKF, showing increased hydroperoxide production in the samples exposed to UV light and lower hydroperoxide production in the emulsified MKF samples. The conjugated diene value also proved the aforementioned, although the activity was very low due to the low PUFA content of MKF. It was clear that a higher linoleic acid content in the fat will necessarily lead to more conjugated dienoic shifts during the first stages of oxidative deterioration. The secondary stage of oxidation, measured by the *p*-anisidine value, indicated a higher production of 2,4-dienal and 2-alkenal in oils with elevated levels of PUFA. The AV also correlated well with the PV, indicating a rise in secondary oxidation products as the primary products were broken down. Very small amounts of secondary products were produced by all the MKF samples. The highest AV values were obtained from the samples exposed to UV light, while the lowest values were obtained from the emulsified samples. A good correlation was found between the AV and PV values of MKF.

The total oxidation was a good indication of the influence of the fatty acid profile on oxidative stability and the high PUFA oils were once again more oxidised

than the MKF. Canola oil was shown to oxidise very little and it can be assumed that the low values were due to the freshness of the sample obtained from the supermarket and the relatively low temperature of storage. The influence of storage conditions on oxidative stability was clear from the samples exposed to UV light, which showed significantly higher oxidation than the crude and emulsified samples, with emulsification being the most stabilising treatment.

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CHAPTER 7

PREDICTION OF THE OXIDATIVE STATUS OF MANGO KERNEL FAT (*Mangifera indica* L.) USING FOURIER TRANSFORM NEAR INFRARED SPECTROSCOPY

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Summary

A Fourier transform near infrared (FT-NIR) spectrophotometer was employed to record the spectra of 300 samples of crude, cold pressed mango kernel fat (MKF), using one-third for independent validation. Samples were scanned at 40-day intervals to detect spectral differences related to the oxidative changes in MKF over a period of 240 days and correlated with the following reference methods: The peroxide value (PV), conjugated diene (CD) value, *p*-anisidine value (AV) and changes in fatty acid composition as measured by gas chromatography (GC). Spectral data was manipulated using both The Unscrambler 6.11 (CAMO ASA) and Simca-P 8.1 (Umetrics AB) software packages. Principal component analysis (PCA) indicated classification between 0, 40 and remaining (80 – 240) days. Partial least squares (PLS) regression yielded the best models in all cases. The best calibration model for PV was developed using the raw data and had a standard error of prediction (SEP) of 0.46 meq.kg^{-1}, correlation coefficient (r) of 0.95, bias of 0.02 and root mean square error of prediction (RMSEP) of 0.46 meq.kg^{-1}. The CD calibration model had a r of 0.90, SEP of 0.01%, bias of 0.001 and RMSEP of 0.01% when using the raw data set, with similar values for derived data sets (Savitsky-Golay 1st and 2nd derivatives). Clustering was evident in these scatter plots, separating the observations at 40 days from the rest. The best calibration model for the AV was obtained with the 2nd derivative pre-processing after the exclusion of one outlier and had a SEP of 0.32 mmol.kg^{-1}, bias of 0.03, RMSEP of 0.32 mmol.kg^{-1} and correlation coefficient of 0.92. The C18:2 model was built using the Savitsky-Golay 1st derivative and yielded a RMSEP of 0.32%, bias of 0.05, r of 0.82 and SEP of 0.31%, which compared well with the calculated SEL of 0.25%. The C18:3 model had a SEP of 0.05%, bias of 0.01, r of 0.54 and RMSEP of 0.05%. The calibrations referenced against C18:1 (*cis*), C18:0 and C16:0 yielded weaker correlations, which probably were due to the stability of these fatty acids when compared to the spectral variation. The ratios between C18:2/C18:0 and C18:2/C16:0 were best described by building calibration

models using no pre-processing. The C18:2/C18:0 model had a SEP of 0.02%, RMSEP of 0.02% and r of 0.72. The C18:2/C16:0 model yielded a SEP of 0.05%, RMSEP of 0.05% and correlation coefficient of 0.70.

Introduction

The determination of oxidative stability and oxidation products is essential to determine the shelf life, acceptability and nutritional quality of edible oils (Gunstone, 1996). As lipid oxidation occurs in different stages, its measurement involves a variety of techniques. The peroxide value (PV) is most commonly used in the quality control of edible oils and determines the primary oxidation products. Another method to determine the early stages of oxidation is the conjugated diene value (CD), which measures the conjugation formed as the unsaturated lipid oxidises (Nawar, 1996). The *p*-anisidine value (AV) is a widely accepted method to determine secondary oxidation products, by estimating the level of aldehydes, primarily 2-alkenals, present in the oil.

The PV, CD and AV methods are accurate in providing an indication of the oxidation status of an oil, but are time consuming, costly, destructive to the sample and require large amounts of glassware and potentially hazardous chemical reagents. In order to decrease the disadvantages of the analytical methods, alternative methods have evolved to measure lipid oxidation (Yildiz *et al.*, 2001). One of these methods is the application of Fourier transform near infrared spectroscopy for the prediction of the oxidative status of edible oils.

Unsaturated fatty acids present in these oils (especially linoleic acid) play the dominant role in oxidative deterioration as these compounds undergo chemical modification, resulting in the production of hydroperoxides and subsequently, odorous compounds (e.g. 2-alkenals) (Gunstone, 1996). Unsaturation of fatty acids is articulated especially in the near infrared region between 2100 and 2400 nm and more specifically by the stretching vibrations of the =C-H and C=C bonds (2140 nm), the C= and asymetrical stretching vibrations of the CH_2 bonds (2190 nm) and the symmetrical stretching of the CH_2 and deformations of the $=CH_2$ bonds (2347 nm) (Osborne *et al.*, 1993). The spectral variation observed between 1650 and 1850 nm is attributed to the first overtone stretching vibrations of the CH_3 and CH_2 bonds at 1700 nm and 1756 nm respectively. These changes are affected by the CH_2 to CH_3 ratio

and are therefore related to the chain length of the fatty acids. Successful classifications of vegetable oils based on the fatty acid profile have been reported (Hourant *et al.*, 2000). The discriminant functions were based on the absorbance values ranging between the 1700 – 1800 nm and 2100 – 2400 nm regions. No references were found for calibrations correlated to changes in fatty acid concentrations.

Strong correlations have been found between the PV, CD and AV reference data and the NIR predicted values during the development of calibration models relating to the oxidative status of soybean oil (Yildiz *et al.*, 2001). According to Osborne *et al.* (1993), the first and second overtones of –OH stretching for both free and intermolecular H-bonded peroxide can be assigned to wavelength ranges of 1400 – 1560 nm and 950 – 1040 nm respectively. Yildiz *et al.* (2001) found that the most influential wavelengths for measuring CD content with a 1 mm cuvette ranged from 2300 to 2500 nm when using a forward stepwise multiple linear (FSML) regression. With PLS regression, however, it was found that the best region for predicting CD with a 2 mm path length was 1100 – 2200 nm (Yildiz *et al.*, 2001). This corresponds with the –OH stretching first overtone (1400 nm) and the CH stretching second overtone (1200 nm) (Osborne *et al.*, 1993). Including the region beyond 2200 nm did not improve the results for the PLS model when using a 1 mm cuvette. According to Tompkins & Perkins (1999), the *p*-anisidine value has a low correlation (0.33) with nonanal production. Carbonyl compounds, such as 2-alkenals or nonanal, do not have absorption bands in the NIR region (Yildiz *et al.*, 2001). This implies that NIR calibrations would correlate the variation in such compounds to the variation associated with compounds that can be measured in the NIR region. This causes difficulties during the development of NIR models for predicting the *p*-anisidine value of edible oils.

The free fatty acids and level of unsaturation of fats and oils have been determined with infrared spectroscopy (IR) (Sato *et al.*, 1991). Fourier transform infrared spectroscopy (FT-IRS) has also been used in the determination of PV and *cis* and *trans* fatty acid contents of fats and oils (Van de Voort *et al.*, 1994). Near infrared (NIR) spectroscopy has been used as an authenticity-testing tool to differentiate among vegetable oil types (Bewig *et al.*, 1994) as well as in determination of the free fatty acids (FFA), iodine value, and the fatty acid composition of fats and oils (Sato *et al.*, 1991; Che Man & Moh, 1998).

The aim of this study was to predict the oxidative status of mango kernel fat (MKF) with Fourier transform near infrared spectroscopy (FT-NIRS), using the peroxide, conjugated diene and *p*-anisidine values, as well as the changes observed in fatty acid profile as measured by gas chromatography over 240 days, as reference values.

Materials and methods

Samples

Three individual batches crude, cold-pressed, mango kernel fat (MKF) were obtained from Specialised Oil cc. (Industria road 2, Industria, Louis Trichardt 0920, South Africa). The clean mango stones were collected from a fruit juice plant (Valley Farms, P.O. Box 163, Levubu 0929, South Africa), followed by manual decortication using a knife. The mango kernels were dried in an electronically controlled oven for 24 hours at 60°C until a moisture content of 12.5% was reached. The dried kernels were then stored at room temperature for three to six days and mechanically pressed at 45°C. The fat was not refined or bleached.

The MKF was stored in 25 ml Chromacol clear glass bottles. Prior to filling, the oil was liquefied at 45°C. This was done to ensure the liquid state of the fat during the entire filling time of the bottles before re-solidification of the fat occurred. Headspace was allowed in 50% of the samples by filling the bottles with approximately 16 ml of the fat. The other half of the samples were filled to the top with fat and closed with a Teflon cap to ensure total oxygen exclusion.

Duplicate samples were stored in the dark at 5, 15, 25 and 40°C for 240 days for reference methods and NIR scanning respectively. Adequate vials for every oil sample were subjected to each storage condition so that no vial had to be reused once it had been removed from storage and used for analyses.

Reference methods

At 40-day intervals, samples from all three batches were liquefied for 10 minutes at a temperature of *ca.* 45°C. This was necessary as MKF was still solidified at 25°C. This led to an easily obtainable, homogenous liquid sample. The samples were shaken lightly and opened just before analysis. Analyses were done in duplicate for all batches.

The peroxide value was determined according to the American Oil Chemists Society (AOCS) Method Cd 8 53 (1985). Sample sizes were, however, reduced to 0.5g ± 0.0009, using 100 ml instead of 250 ml Erlenmeyer flasks. Volumes were reduced to 10 ml of the acetic acid-chloroform solution, 10 ml of distilled water and a 0.01 M sodium thiosulfate titrant. The conjugated diene value (CD) was determined according to the AOCS Method Ti la-64 (1993). Sample sizes were reduced to 18 ml, diluting to volume with hexane in a 25 ml volumetric flask. The p-anisidine value was determined according to the AOCS Method Cd 18-90 (1992). The absorbances at 233 nm for CD and at 350 nm for AV were measured with a Phillips PU 8700 series, UV/Visible spectrometer (Phillips Scientific and Analytical Equipment). The total oxidation (TOTOX) value was calculated by the formula: TOTOX = 2PV + AV. Gas chromatography was done according to the method described in chapter 3.

Fourier transform near infrared measurements

At 40-day intervals, samples were removed from 5, 15, 25 and 40°C incubation and liquefied at *ca.* 45°C for 10 minutes. After liquefaction, the bottles were shaken lightly and opened just before presentation to the instrument.

Spectra were generated using a Perkin Elmer Spectrum IdentiCheck FT-NIR spectrophotometer and Spectrum IdentiCheck software (version 3.02.01). The oil was kept in a liquified state during scanning. Using an Eppendorf Research 200 pippette, a 0.2 mm quartz Suprasil cuvette (Helma) was filled and presented to the instrument.

Samples were aligned in the beam using the Perkin Elmer sample slide accessory with cuvette holder. Different resolutions, ranging between 4 cm^{-1} and 64 cm^{-1}, were tested to determine the most appropriate setting for this study. Transmission absorbance spectra were measured over the wavelength range of 1100 nm – 2500 nm at 2 nm intervals (701 data points). In between samples of different batches, cuvettes were cleaned with warm water and Extran MA O3 phosphate-free soap (MERCK).

Calibration development

Spectral data was manipulated using both The Unscrambler 6.11 (CAMO ASA) and Simca-P 8.1 (Umetrics AB) software packages. Calibrations were developed from a

total data set of 300 samples of which approximately one-third, covering the same variation and reference range as that of the calibration set, was used for independent validation. Principal component analysis (PCA) was performed on the raw, 1st and 2nd derivative data sets. The derivation was done using The Unscrambler software, while PCA was done using Simca-P. Partial least squares (PLS) and principal component regression (PCR) analyses were evaluated to determine the optimum model. Both the raw data and derived data were employed to build calibrations and spectral values were derived using Savitsky-Golay derivatives.

For each model, performance indicators such as standard error of prediction (SEP) (Formula 7.1), root mean square error of prediction (RMSEP) (Formula 7.2), bias and coefficient of correlation (r) (Formula 7.3) were used to determine the most accurate calibration. In some cases, the SEP values were compared to the calculated standard error of laboratory (SEL) (Formula 7.4) values for reference methods to evaluate predictive ability.

$$SEP = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i - bias)^2}{n-1}} \qquad \text{...7.1}$$

where $\hat{y}_i$ = predicted property of the i^{th} standard of the independent validation sample set

y_i = actual property of the i^{th} standard

n = number of spectra

$bias$ = the average differences between reference and predicted values

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n-1}} \qquad \text{...7.2}$$

$$r_{jk} = \frac{\sum x_{ij} x_{jk}}{\sqrt{\left(\sum x_{ij}^{\,2} \sum x_{jk}^{\,2}\right)}} \qquad \text{...7.3}$$

where x_{ij} = absorbance value (nm) of the i^{th} wavelength for spectra j

x_{jk} = absorbance value (nm) of the i^{th} wavelength for spectra k

$$SEL = \sqrt{\frac{\sum (y_1 - y_2)^2}{2n}} \qquad \text{...7.4}$$

where y_1 and y_2 = values of duplicate determinations

n = number of samples analysed

Results and discussion

The mean spectrum (n = 300) of mango kernel fat is shown in Figure 7.1. Variation was expected in the wavelength regions ranging from 1650 nm to 1850 nm and 2100 nm to 2400 nm (Figure 7.2). Changes in this region are affected by the CH_2 to CH_3 ratio as well as the stretching vibrations of the =C-H and C=C bonds and are therefore related to the chain length and unsaturation of the fatty acids (Osborne *et al.*, 1993). As the oxidative deterioration of lipids is directly linked to alterations within the double bonds occurring in unsaturated fatty acids, these were identified as the regions to monitor during the shelf life study of mango kernel fat. Oxidative changes over time as a result of changes in unsaturated fatty acids in the mango kernel fat can be appropriated to the C18:2 and C18:3 (trace) fatty acids.

A high level of smoothing was observed over the whole region when a resolution of 64 cm^{-1} was used, while at 32 cm^{-1} some spectral information was lost in the region of 2350 – 2370 nm (Figure 7.3). Resolutions of 4, 8 and 16 cm^{-1} were equally representative of the activity in the samples with 16 cm^{-1} being the most time effective resolution.

PCA illustrates pure spectral differences between samples analysed at 0, 40 and remaining days (Figures 7.4, 7.5 and 7.6). This classification was expected to correlate well with the oxidative changes (hydroperoxide formation and decomposition) observed during the reference analyses.

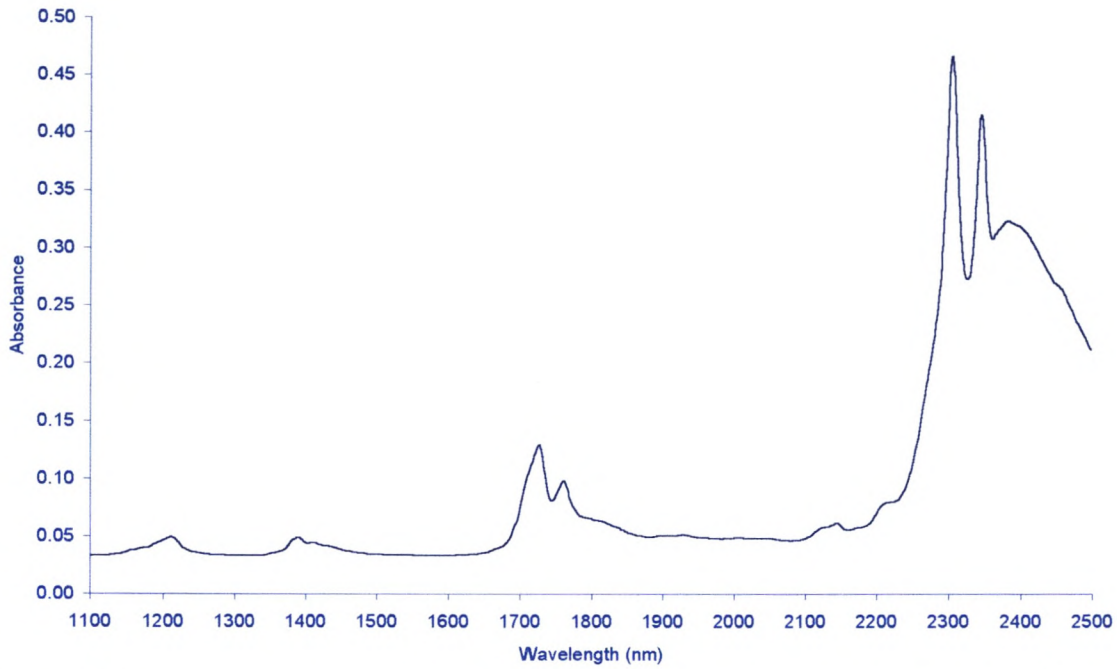

Figure 7.1. Mean spectrum of mango kernel fat scanned at a resolution of 16 cm^{-1}.

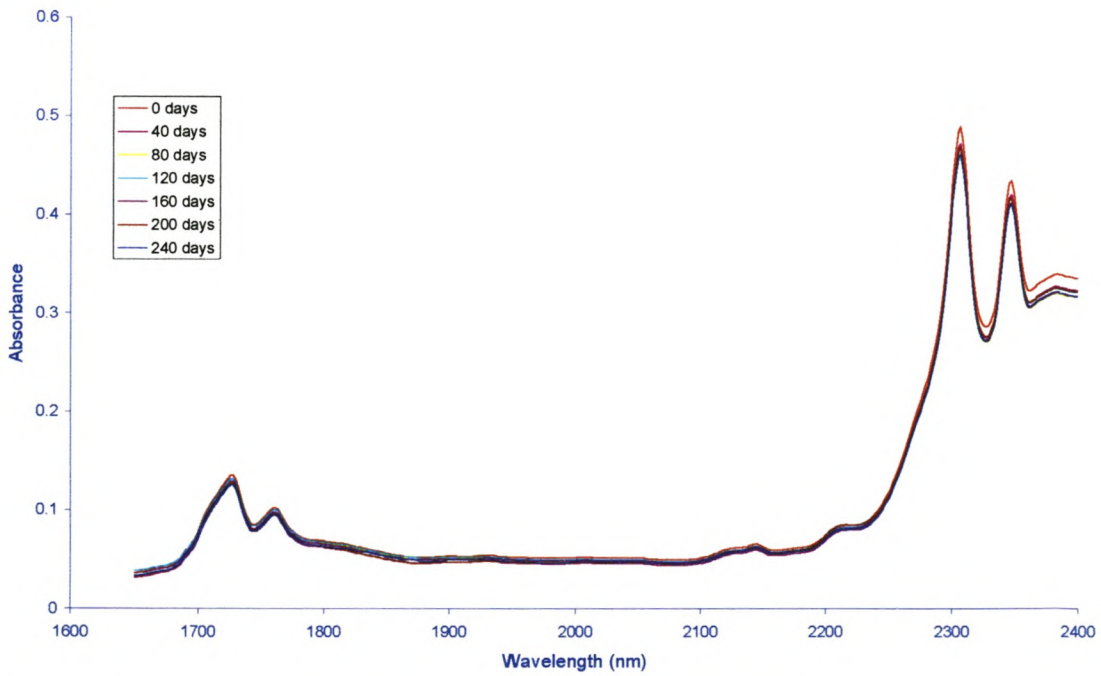

Figure 7.2. Spectral variation between representative samples from 0 to 240 days.

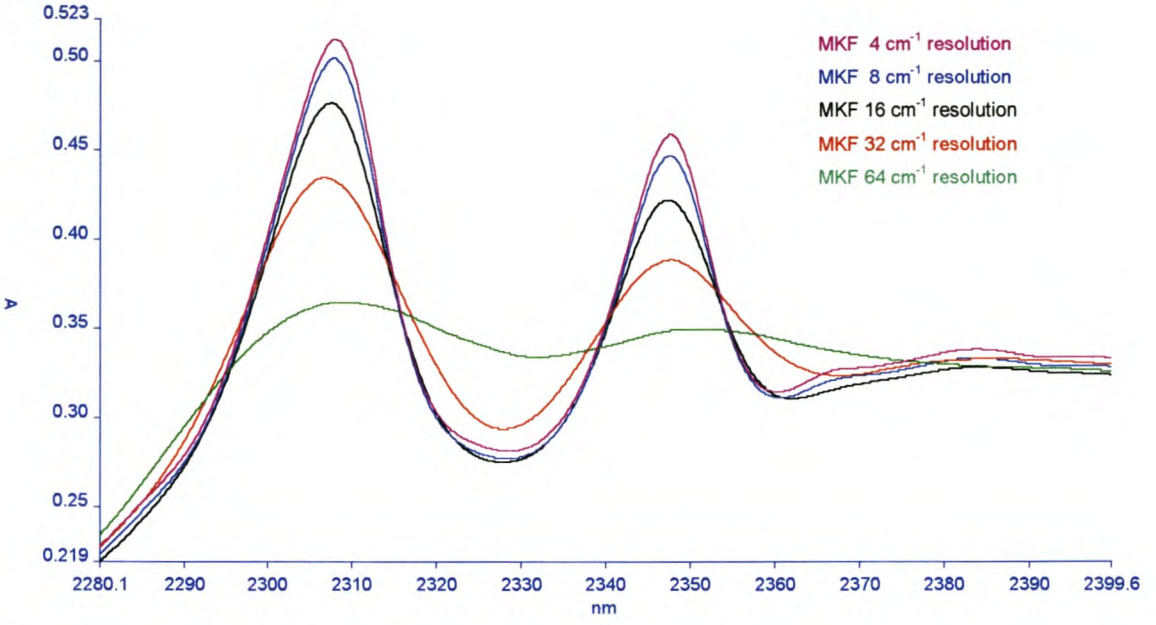

Figure 7.3. Comparison of FT-NIR spectra of mango kernel fat at different resolutions.

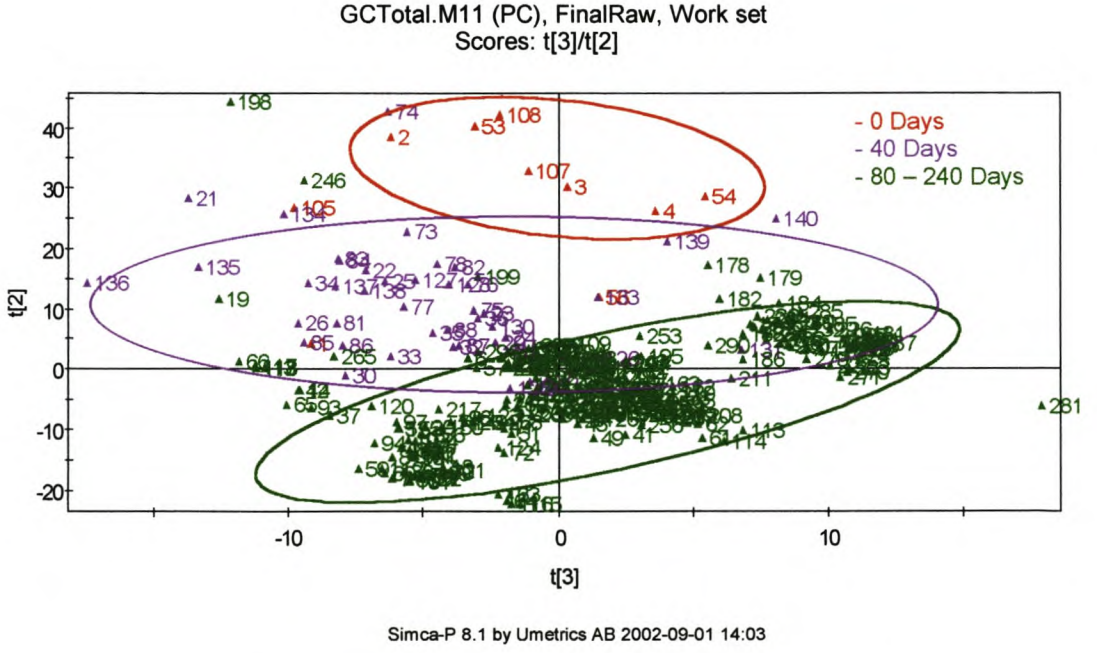

Figure 7.4. PCA plot of raw spectral data of crude, cold-pressed mango kernel fat.

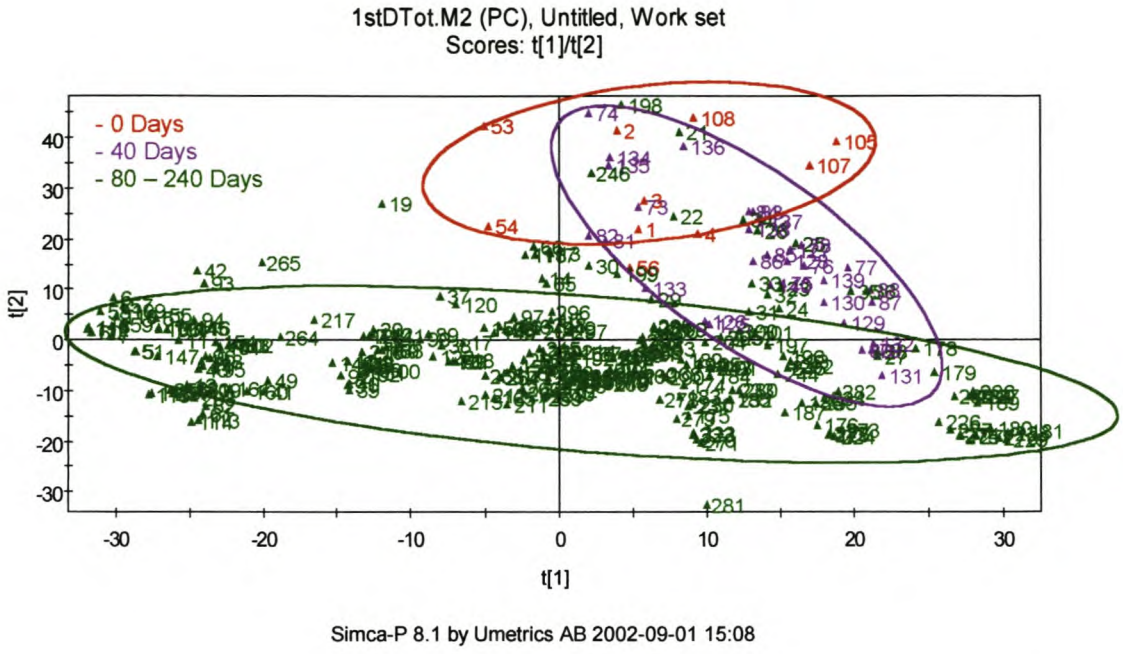

Figure 7.5. PCA plot of the derived data (Savitsky-Golay 1st) of mango kernel fat.

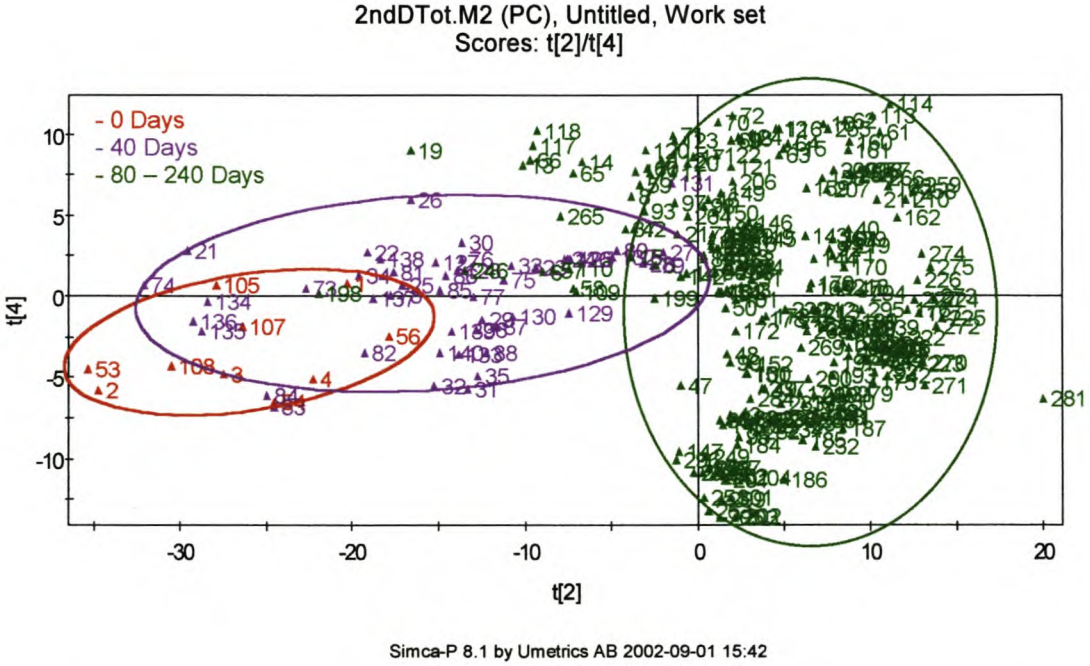

Figure 7.6. PCA plot of the derived data (Savitsky-Golay, 2nd) of mango kernel fat.

Peroxide value

The peroxide value (PV) of fats and oils indicates the level of oxidation and rancidity, expressed as hydroperoxide production, measured in meq.kg^{-1} oil. The PV of mango kernel fat never exceeded 5 meq.kg^{-1} when in contact with a limited amount of enclosed oxygen and stored at 5, 15, 24 and 40°C over a period of 240 days, indicating that only a minimal amount of oxidation occurred (Figure 7.7). According to Osborne *et al.* (1993), the first and second overtones of –OH stretching for both free and intermolecular H-bonded peroxide can be assigned to wavelength ranges of 1400 – 1560 nm and 950 – 1040 nm respectively. Although only small variations between the samples are visible in this region, the calibration of NIR data often involves explaining such minimal fluctuations. The regression model was also developed by combining the total variation across all data points. During calibration the best fit for the regression line was found by using a partial least squares (PLS) regression (Figure 7.8). Furthermore it was established that the raw (unprocessed) data delivered the best results. An independent validation set (n = 97), excluding two outliers, was selected to represent the PV values between 0 and 5 meq.kg^{-1}. The SEP for this model was 0.46 meq.kg^{-1}, with a correlation of 0.95, a bias of 0.02 and a root mean square error of prediction (RMSEP) of 0.46 meq.kg^{-1} (Table 7.1). A total of 13 PLS factors or principal components (PCs) were required. No improvement in the predictive ability of the model was attained when the Savitsky-Golay first derivative was used. The residual validation variance plot is depicted in Figure 7.9.

Conjugated dienes

The shifting of double bonds as the 1,4-pentadienoic system is attacked, is depicted in Figure 7.10. These changes have been observed in the 1100 – 2200 nm (Yildiz *et al.*, 2000) and 2200 - 2400 nm wavelength ranges (Osborne *et al.* 1993).

Calibration models for mango kernel fat were built across a wavelenth range of 1100 to 2500 nm using the raw data as well as the Savitsky-Golay first and second derivatives. Values for the raw data showed a correlation coefficient of 0.90, SEP of 0.01%, bias of 0.001 and RMSEP of 0.01% (Table 7.2). Eight PLS factors were required to obtain this model. Similar values, with slight improvements were obtained for both the Savitsky-Golay first and second derivatives (Tables 7.3 & 7.4), with the second derivative showing the best overall values. Validation scatter plots

for the raw, first derivative and second derivative data are illustrated in Figures 7.11, 7.12 & 7.13 respectively. The grouping evident in the lower left-hand corner of all the scatter plots represents the CD values for 0 days (i.e. before any double bond shifting occurred). The residual validation variance plot for the derived data (Savitsky-Golay 2nd) is depicted in Figure 7.14. In accordance with the results of Yildiz *et al.* (2001), a further calibration was developed using only the wavelength range from 1100 – 2200 nm. No major improvement was noted and the scatter plot for the derived data (Savitsky-Golay 2nd) is shown in Figure 7.15. This model had a SEP of 0.01%, correlation of 0.91 and RMSEP of 0.01% (Table 7.5).

p-Anisidine value

The *p*-anisidine values obtained for MKF ranged between 0.5 and 4.0 mmol.kg^{-1} after 240 days of storage at 5, 15, 25 and 40°C (Figure 7.16). These low values can be explained by the limited formation of 2-alkenals due to the low levels of polyunsaturated fatty acids (*ca.* 6% linoleic & 0.5% linolenic) present in the MKF. Calibration models for MKF were built using the raw data and the Savitsky-Golay 1st and 2nd derivatives. The best model was obtained with the 2nd derivative pre-processing, although it was only slightly better than the 1st derivative. The SEP was 0.32 mmol.kg^{-1} after the exclusion of one outlier (Table 7.6). The bias (0.03), RMSEP (0.32 mmol.kg^{-1}) and correlation coefficient (0.93) were all determined using nine PLS factors. The validation scatter plot of the derived data (Savitsky-Golay, 2nd) is shown in Figure 7.17. The residual validation variance plot for the derived data (Savitsky-Golay 2nd) is depicted in Figure 7.18.

Gas chromatography

Gas chromatographs of mango kernel fat, obtained over a period of 240 days indicated a decrease in the C18:2/C16:0 as well as C18:2/C18:0 fatty acid ratios. Stability was observed for oleic acid (C18:1) and the saturated fatty acids, palmitic (C16:0) and stearic (C18:0) acids, while a decrease was observed in C18:2 (Figure 7.19) and C18:3 (trace). In the present study the oxidative changes in individual fatty acids were studied and calibration models were built using PLS regression. One outlier (25°C, 200 days) was removed from all calibrations. The best model for describing the observed decline in C18:2 was obtained using the Savitsky-Golay 1st

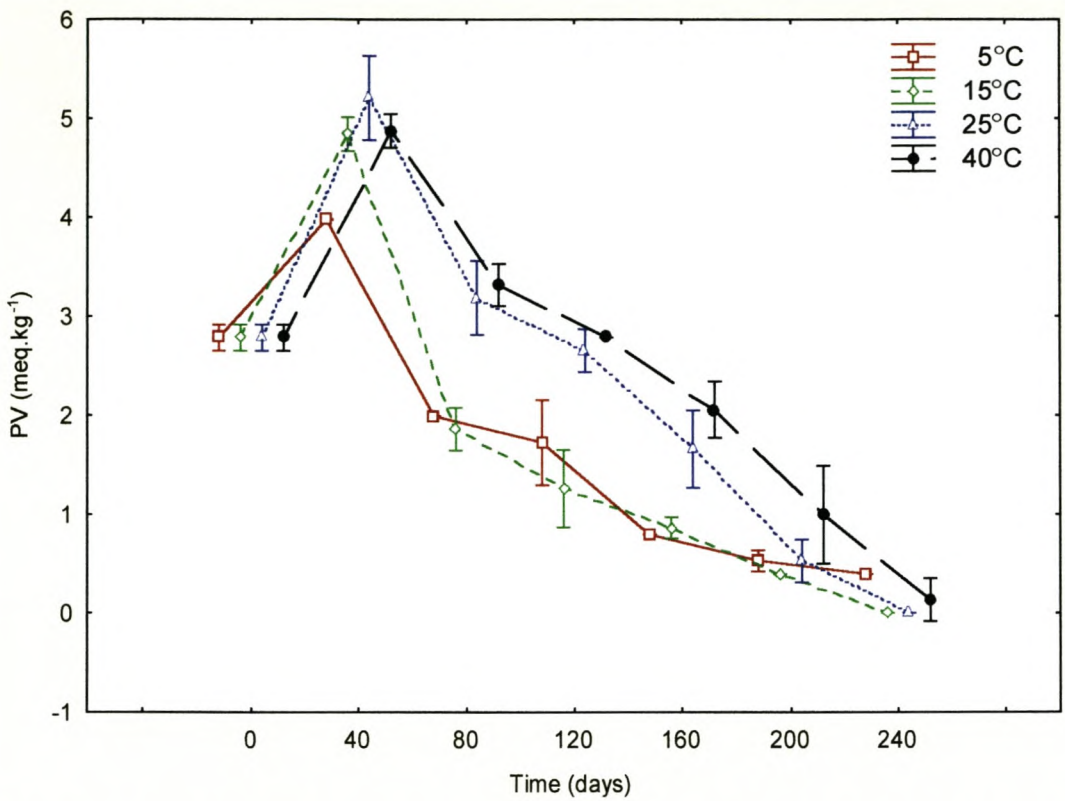

Figure 7.7. Changes in the peroxide value of mango kernel fat stored for 240 days at different temperatures, with a limited amount of oxygen present.

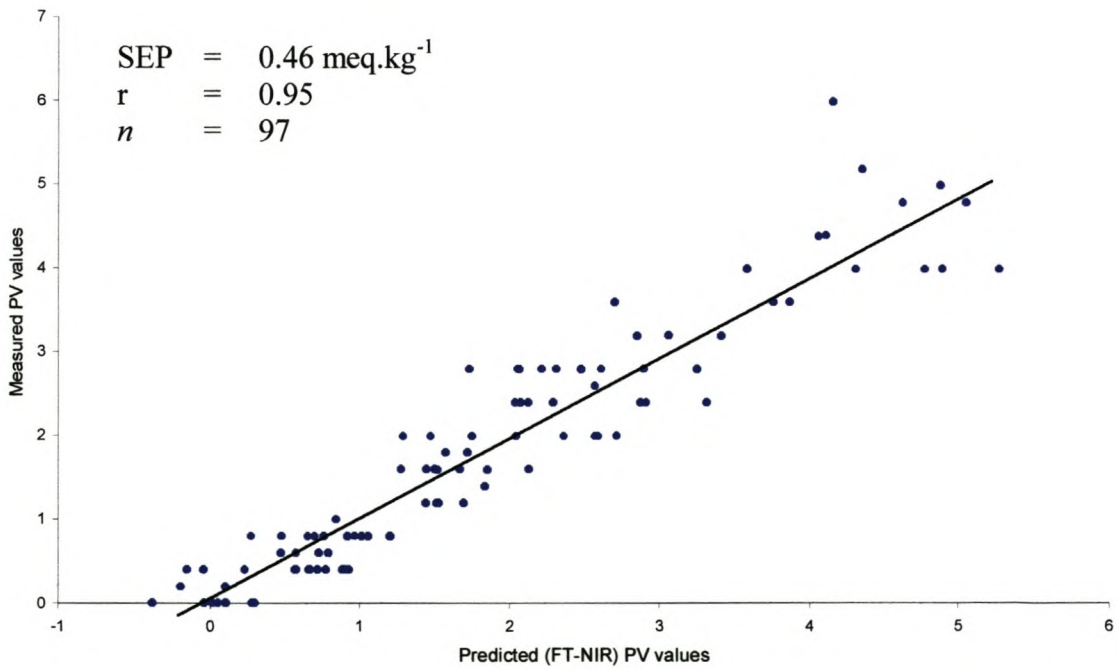

Figure 7.8. A validation plot of the predicted (FT-NIR) peroxide values versus the measured (reference value) peroxide values of mango kernel fat samples over a period of 240 days.

Table 7.1. Statistical results for the peroxide value (PV) calibration.

SEP	0.46 meq.kg^{-1}
Bias	0.02
RMSEP	0.46 meq.kg^{-1}
r	0.95
Elements (*n*):	
Calibration	200
Validation	97
Number of PLS factors	13
Mean	1.77 meq.kg^{-1}
Range	0 – 5.17 meq.kg^{-1}

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

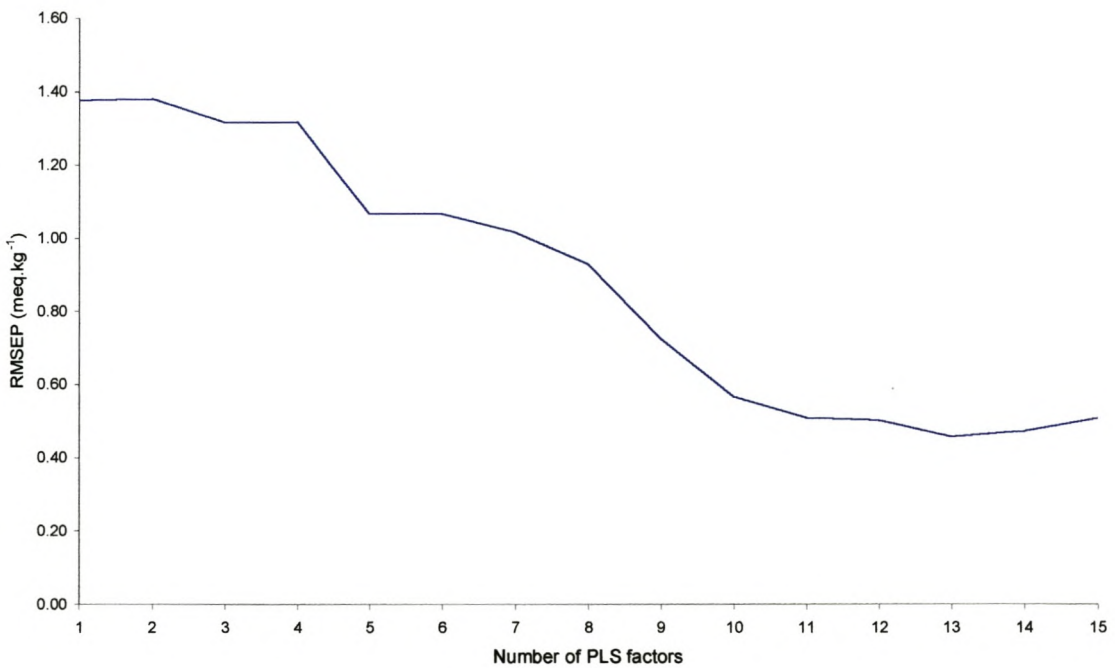

Figure 7.9. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the peroxide value calibration model.

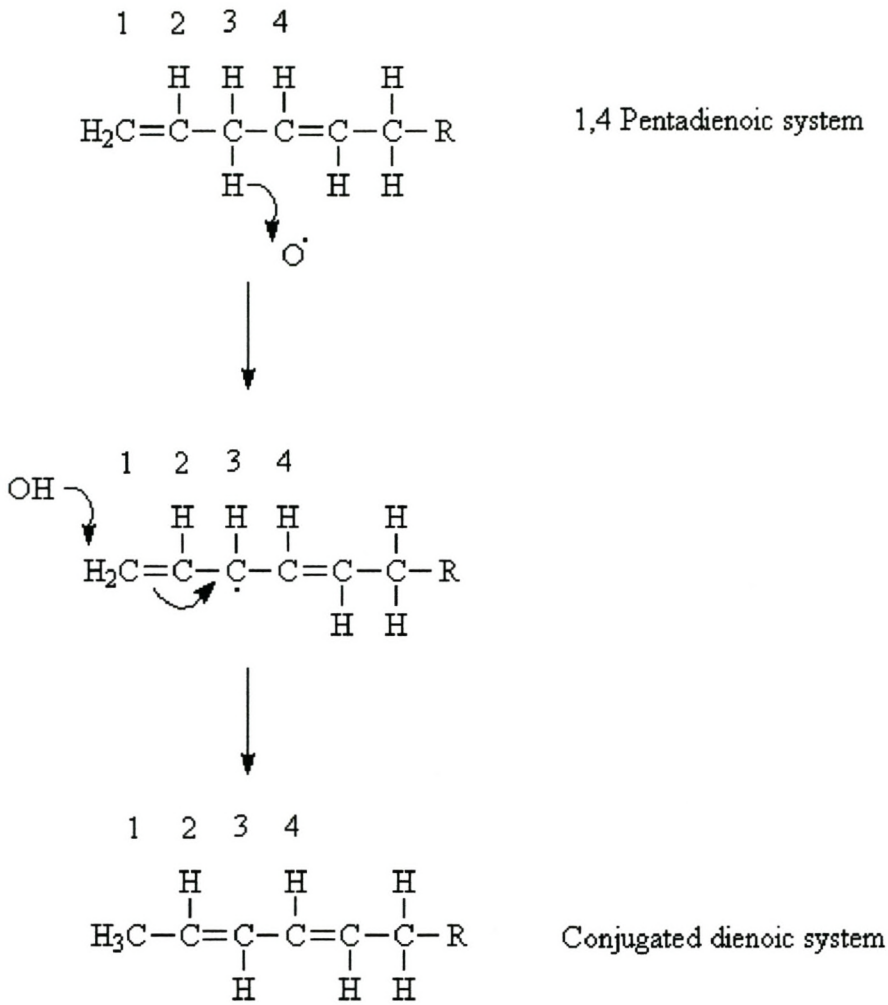

Figure 7.10. Formation of conjugated dienoic bonds by reaction with radical oxygen in a 1,4-pentadienoic system.

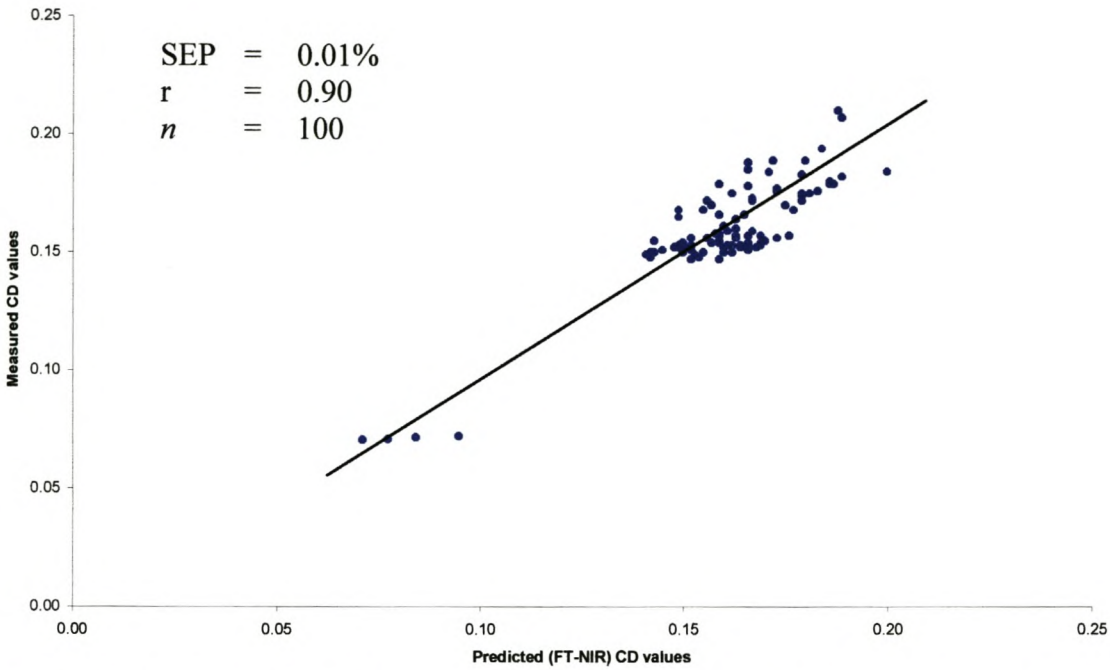

Figure 7.11. A validation plot of the predicted (FT-NIR) conjugated diene values versus the measured (reference value) conjugated diene values of mango kernel fat samples over a period of 240 days (raw data).

Table 7.2. Statistical results for the conjugated diene value (CD) calibration (raw data).

SEP	0.01%
Bias	0.001
RMSEP	0.01%
r	0.90
Elements (*n*):	
Calibration	200
Validation	100
Number of PLS factors	11
Mean	0.16%
Range	0.07 – 0.021%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

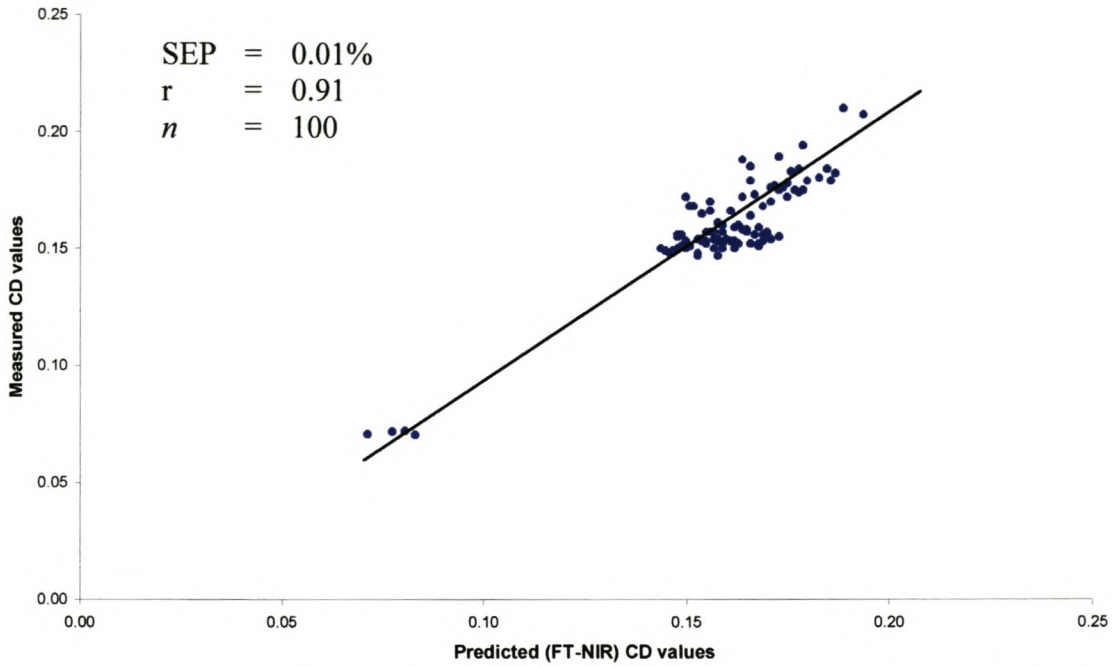

Figure 7.12. A validation plot of the predicted (FT-NIR) conjugated diene values versus the measured (reference value) conjugated diene values of mango kernel fat samples over a period of 240 days (Savitsky-Golay, 1st derivative).

Table 7.3. Statistical results for the conjugated diene value (CD) calibration (Savitsky Golay, 1st derivative).

SEP	0.01%
Bias	0.01
RMSEP	0.01%
r	0.91
Elements (*n*):	
Calibration	200
Validation	100
Number of PLS factors	12
Mean	0.16%
Range	0.07 – 0.21%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

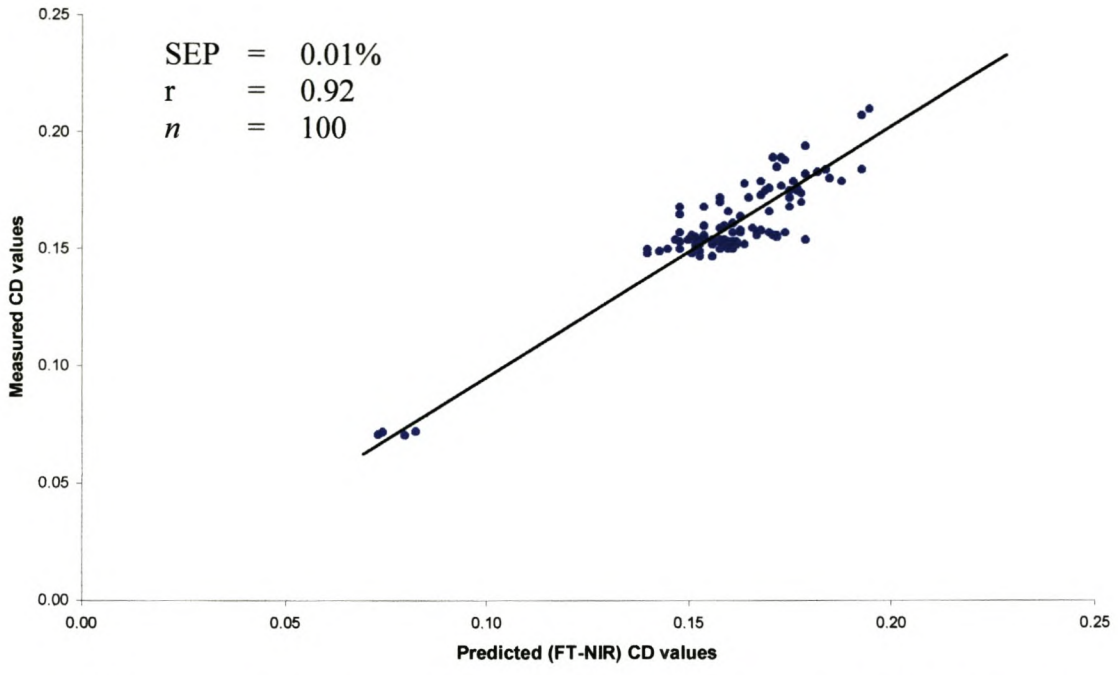

Figure 7.13. A validation plot of the predicted (FT-NIR) conjugated diene values versus the measured (reference value) conjugated diene values of mango kernel fat samples over a period of 240 days (Savitsky-Golay, 2nd derivative).

Table 7.4. Statistical results for the conjugated diene value (CD) calibration (Savitsky-Golay, 2nd derivative).

SEP	0.01%
Bias	0.001
RMSEP	0.01%
r	0.92
Elements (*n*):	
Calibration	200
Validation	100
Number of PLS factors	9
Mean	0.16%
Range	0.07 – 0.21%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

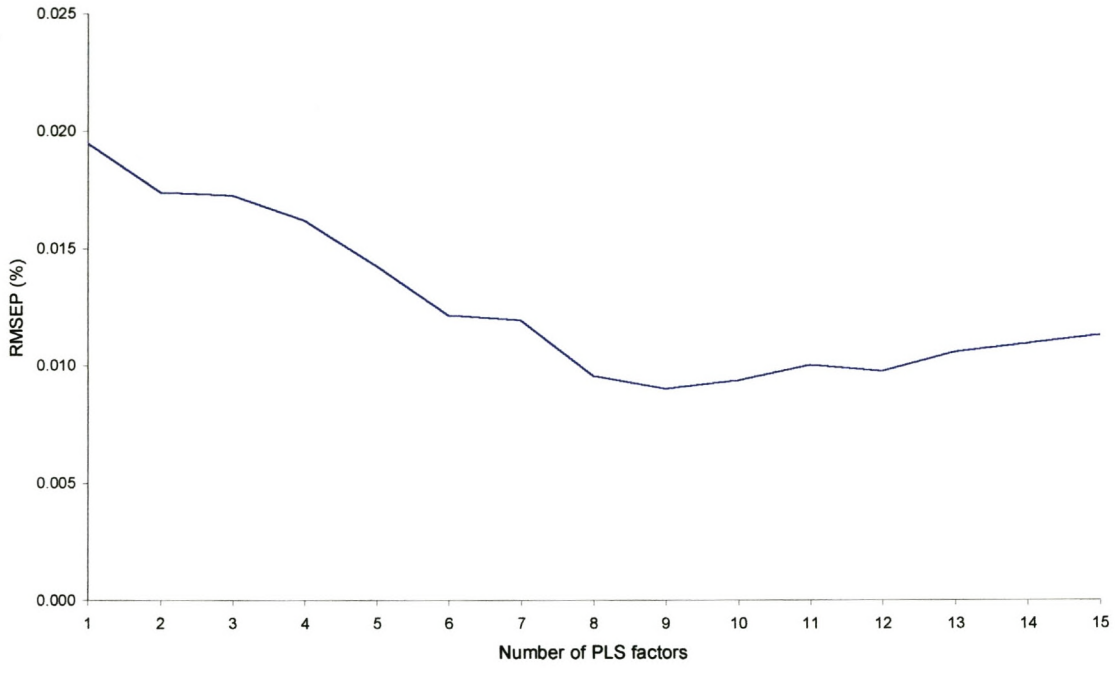

Figure 7.14. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the conjugated diene value calibration model.

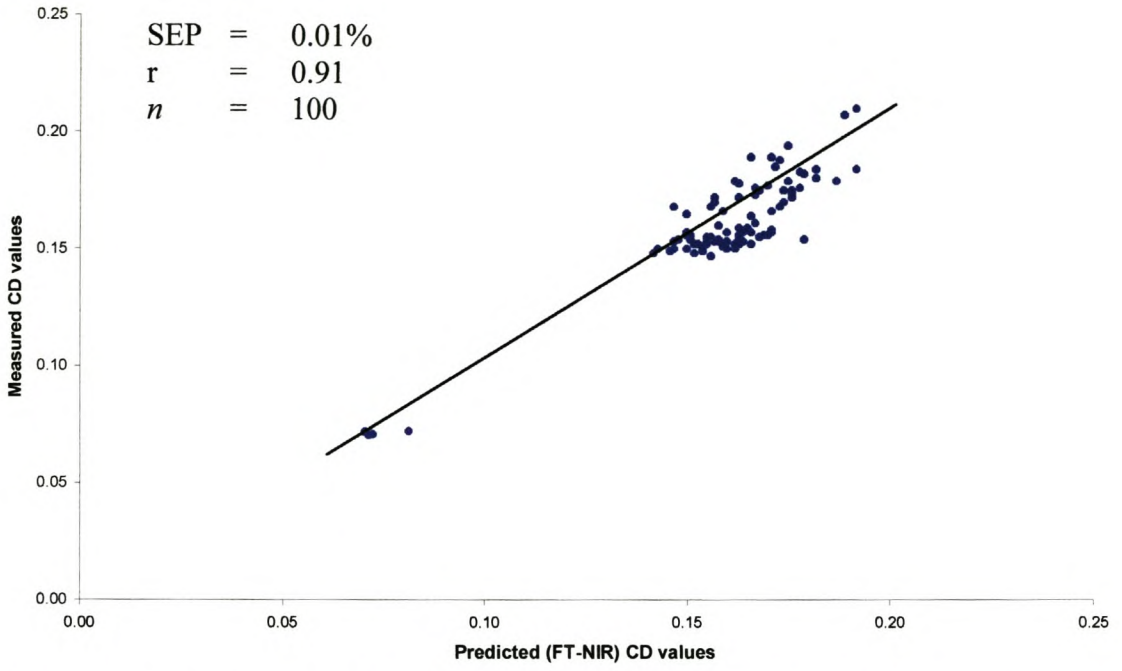

Figure 7.15. A validation plot of the predicted (FT-NIR) conjugated diene values versus the measured (reference value) conjugated diene values of mango kernel fat samples for the wavelength range 1100 nm – 2200nm over a period of 240 days (Savitsky-Golay, 2nd derivative).

Table 7.5. Statistical results for the conjugated diene value (CD) calibration (Savitzky-Golay, 2nd derivative) across the wavelength range 1100 nm – 2200 nm.

SEP	0.01%
Bias	0.001
RMSEP	0.01%
r	0.91
Elements (*n*):	
Calibration	200
Validation	100
Number of PLS factors	8
Mean	0.16%
Range	0.07 – 0.21%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

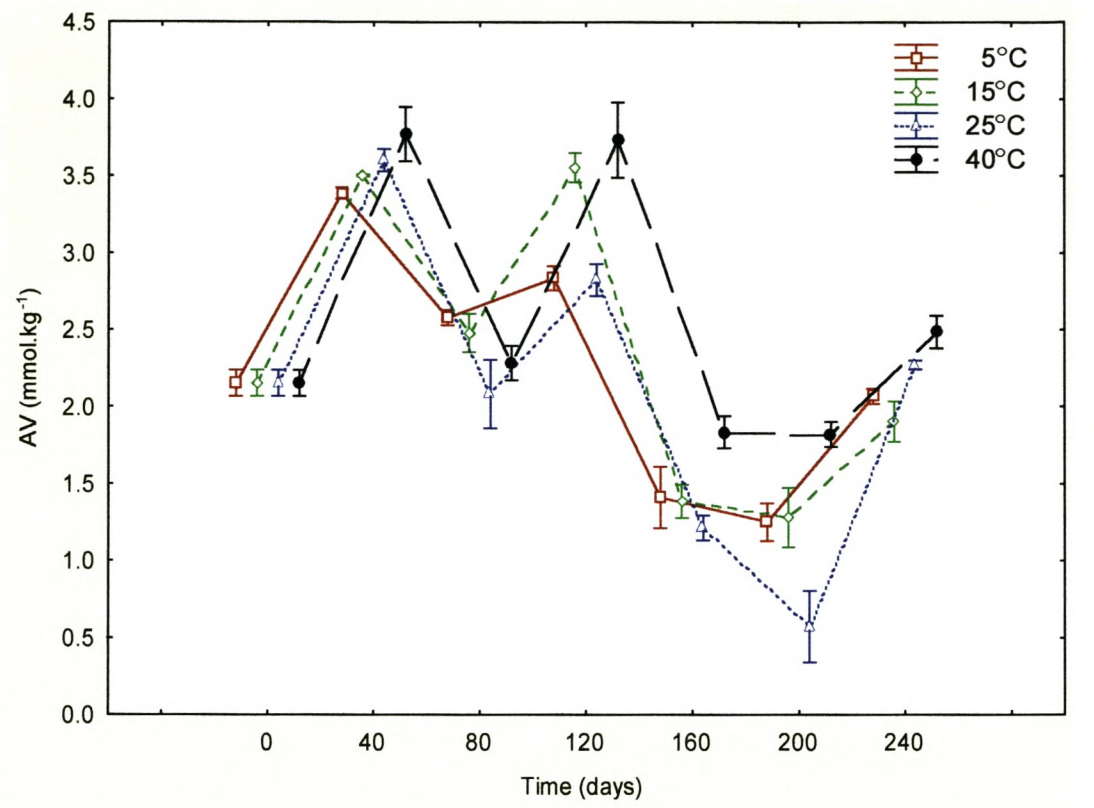

Figure 7.16. Changes in the *p*-anisidine value of mango kernel fat stored for 240 days at different temperatures, with a limited amount of oxygen present.

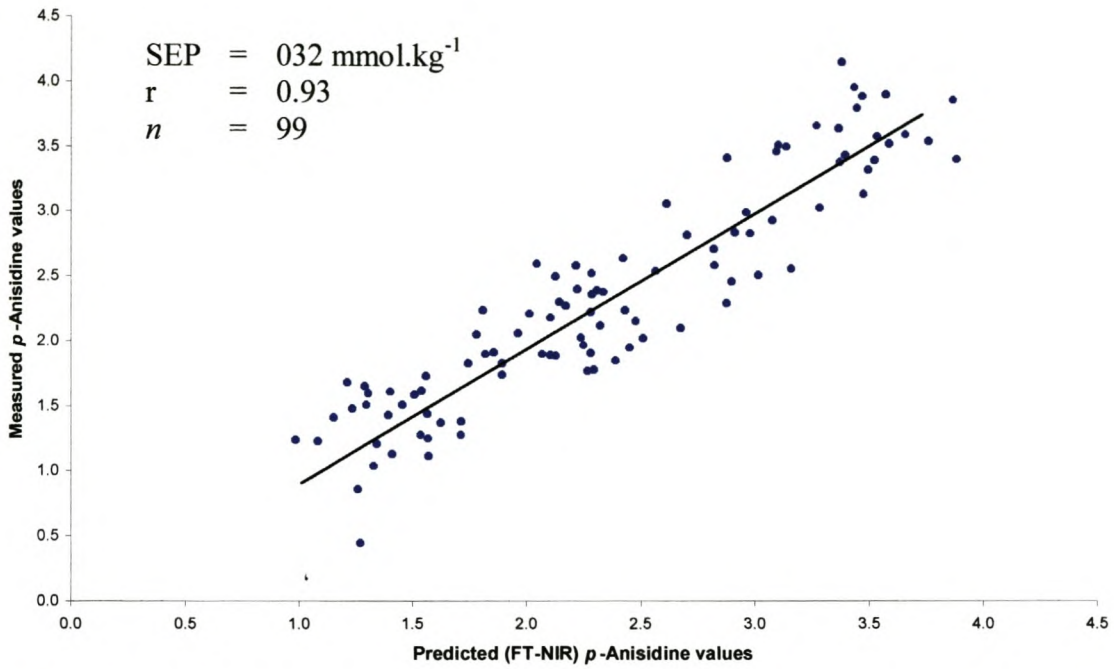

Figure 7.17. A validation plot of the predicted (FT-NIR) *p*-anisidine values versus the measured (reference value) *p*-anisidine values of mango kernel fat samples over a period of 240 days (Savitsky-Golay, 2nd derivative).

Table 7.6. Statistical results for the *p*-anisidine value (AV) calibration (Savitsky-Golay, 1st and 2nd derivative).

	1st derivative	2nd derivative
SEP	0.33 mmol.kg^{-1}	0. 32 mmol.kg^{-1}
Bias	0.02	0.03
RMSEP	0.33 mmol.kg^{-1}	0.32 mmol.kg^{-1}
r	0.92	0.93
Elements (*n*):		
Calibration	200	200
Validation	99	99
Number of PLS factors	9	9
Mean	2.31 mmol.kg^{-1}	2.31 mmol.kg^{-1}
Range	0.41 – 4.15 mmol.kg^{-1}	0.41 – 4.15 mmol.kg^{-1}

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

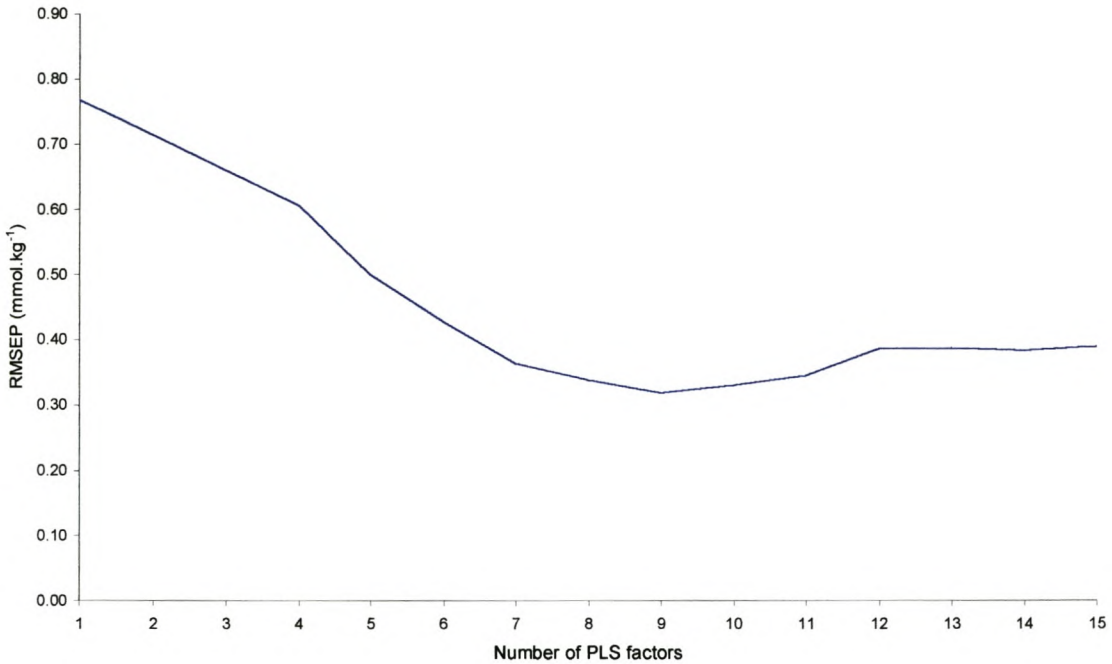

Figure 7.18. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the *p*-anisidine value calibration model.

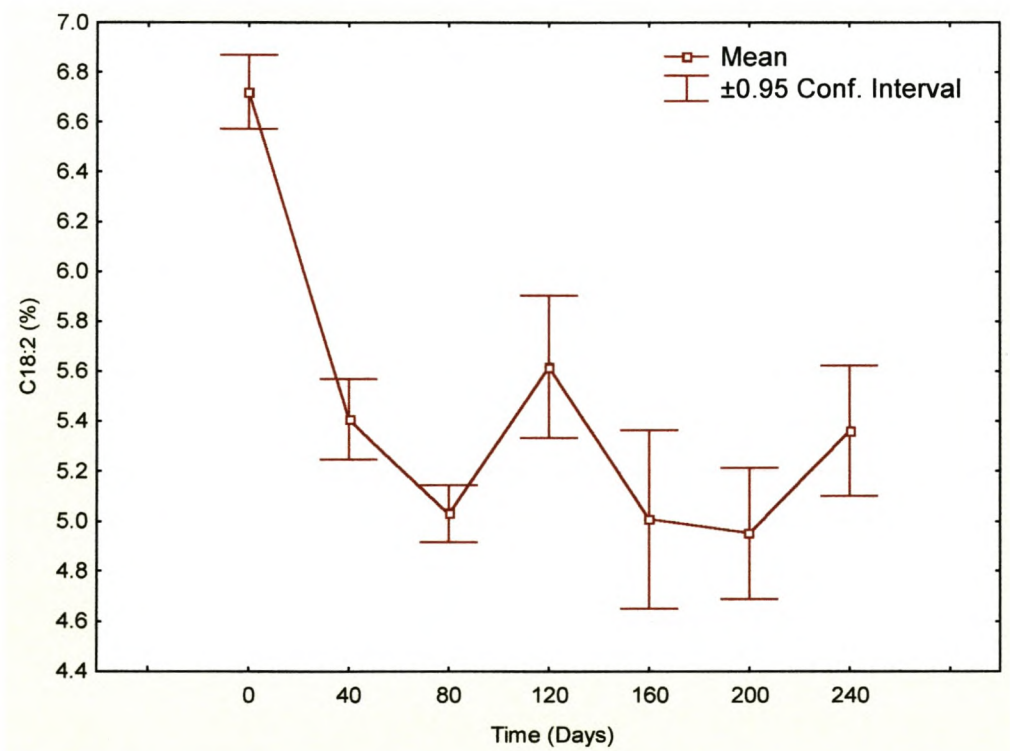

Figure 7.19. Effect of storage over 240 days on on percentage of linoleic acid (C18:2) in mango kernel fat (MKF).

derivative (Figure 7.20). The model had a SEP of 0.31%, RMSEP of 0.32%, bias of 0.05% and r of 0.81 (Table 7.7). This compared well with the calculated SEL of 0.251%. The residual validation variance plot for the derived data (Savitsky-Golay 1st) is depicted in Figure 7.21.

Changes in C18:3 were also best described using the derived data (Savitsky-Golay, 2nd, excluding two outliers) to build a calibration model (Figure 7.22) and a SEP of 0.054%, bias of 0.01, correlation coefficient of 0.54 and a RMSEP of 0.05% was attained (Table 7.8). The low correlation coefficient can be explained by the trace nature of the C18:3. The residual validation variance plot for the derived data (Savitsky-Golay 2nd) is depicted in Figure 7.23. Over-writing after factor 9 is evident from the increase in RMSEP.

Calibrations were also developed for C18:1 (*cis*), C18:0 and C16:0. Weaker correlations were probably due to the stability of these fatty acids in comparison to the spectral variation (Table 7.9). These variations could be caused by changes in the crude fractions of MKF that do not correspond to the slight decreases shown in Figures 3.11 – 3.13 in Chapter 3.

The C18:2/C18:0 and C18:2/C16:0 ratios were best described by building calibration models using no pre-processing (Figures 7.24 & 7.25). One additional outlier was removed for these two calibrations. The C18:2/C18:0 model had a SEP of 0.02%, RMSEP of 0.02% and correlation coefficient of 0.74 (Table 7.10). The C18:2/C16:0 model yielded a SEP of 0.05%, RMSEP of 0.05% and correlation coefficient of 0.70 (Table 7.11). The residual validation variance plots for the C18:2/C18:0 and C18:2/C16:0 models are depicted in Figures 7.26 and 7.27, respectively.

Conclusion

Fourier transform near infrared spectroscopy can be applied successfully in the prediction of the oxidative status of crude, cold pressed mango kernel fat. Clear distinction was made between spectral data generated from mango kernel fat samples analysed on 0, 40 and remaining days (80 – 240). This principal component analysis correlated well with the oxidative changes observed during the shelf life study and represented the un-oxidised oil, formation of hydroperoxides while breaking the double bonds of unsaturated fatty acids and the formation of secondary products

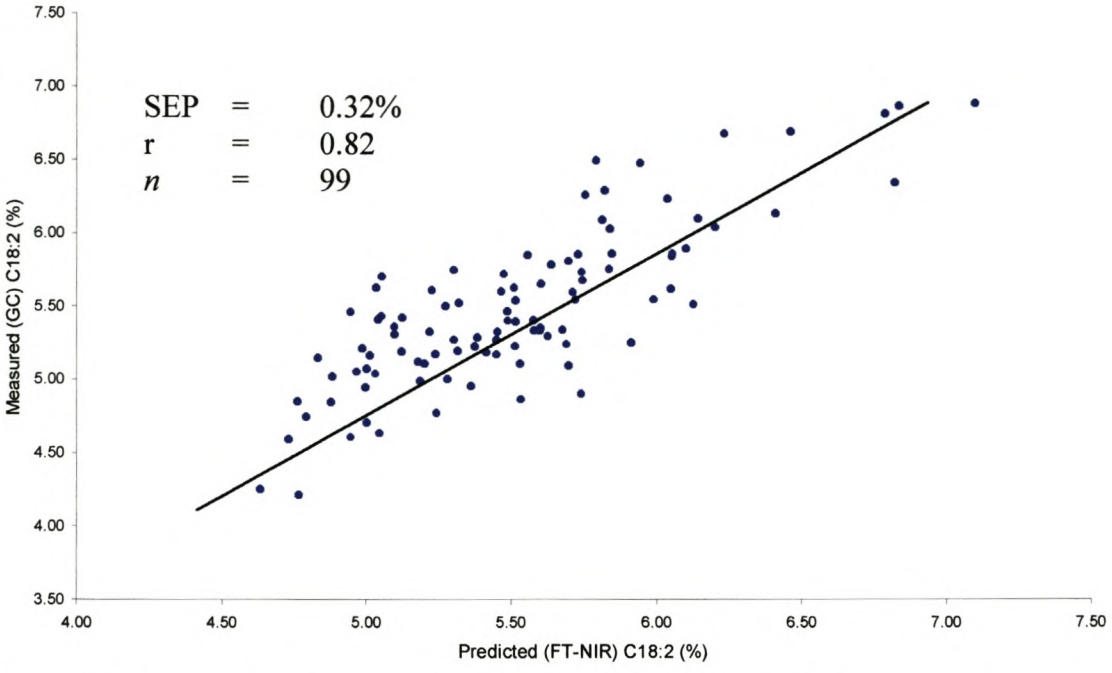

Figure 7.20. A validation plot of the predicted (FT-NIR) C18:2 values versus the measured (reference value) C18:2 values of mango kernel fat samples over a period of 240 days (Savitsky-Golay, 1st derivative).

Table 7.7. Statistical results for the C18:2 calibration (Savitzky-Golay, 1st derivative).

SEP	0.32%
Bias	0.05
RMSEP	0.32%
r	0.82
Elements (*n*)	
Calibration	200
Validation	99
Number of PLS factors	11
Mean	5.47%
Range	3.98 – 6.88%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

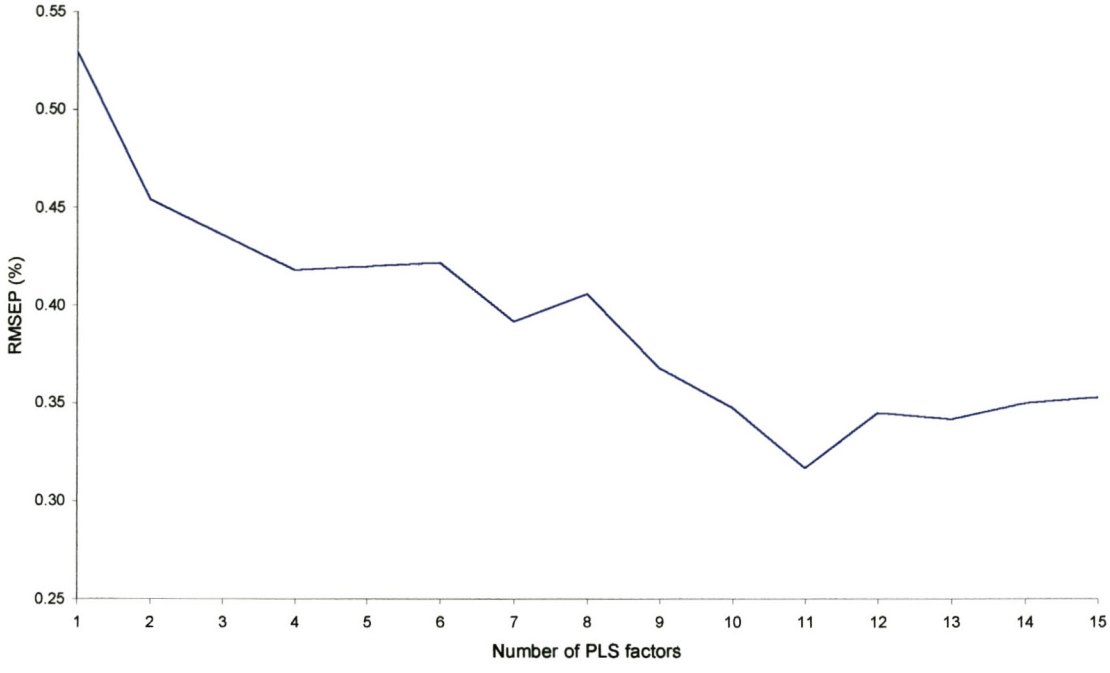

Figure 7.21. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the C18:2 calibration model.

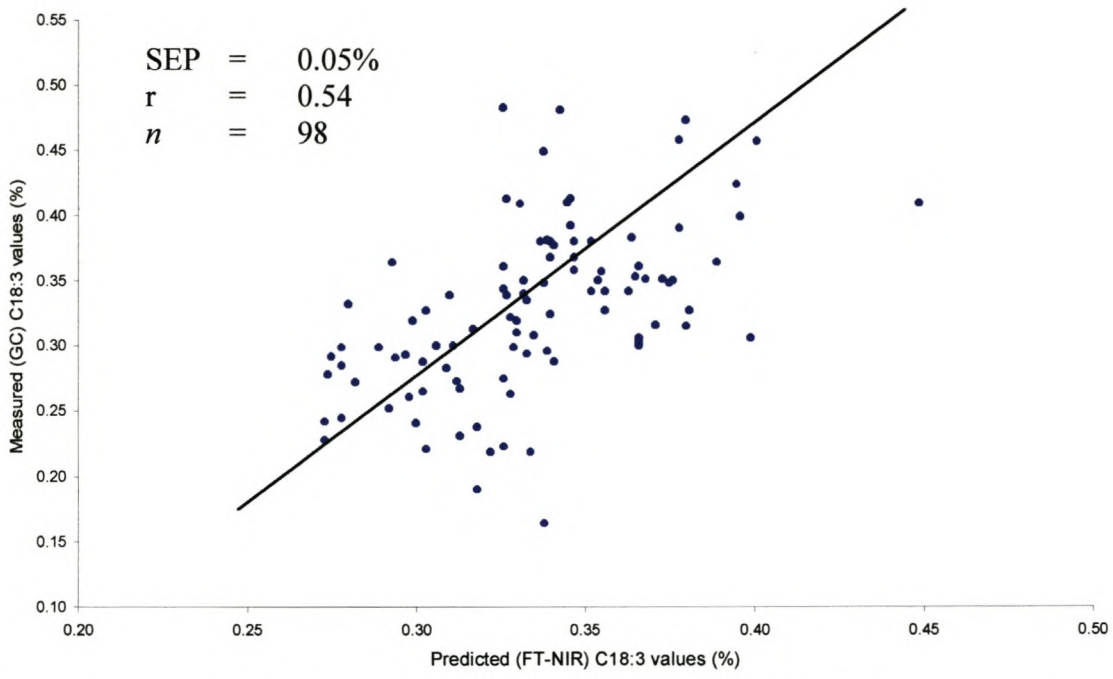

Figure 7.22. A validation plot of the predicted (FT-NIR) C18:3 values versus the measured (reference value) C18:3 values of mango kernel fat samples over a period of 240 days (Savitsky-Golay, 1st derivative).

Table 7.8. Statistical results for the C18:3 calibration (Savitzky-Golay, 1st derivative).

SEP	0.05%
Bias	0.01
RMSEP	0.05%
r	0.54
Elements (*n*):	
Calibration	200
Validation	98
Number of PLS factors	9
Mean	0.33%
Range	0.16 – 0.55%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

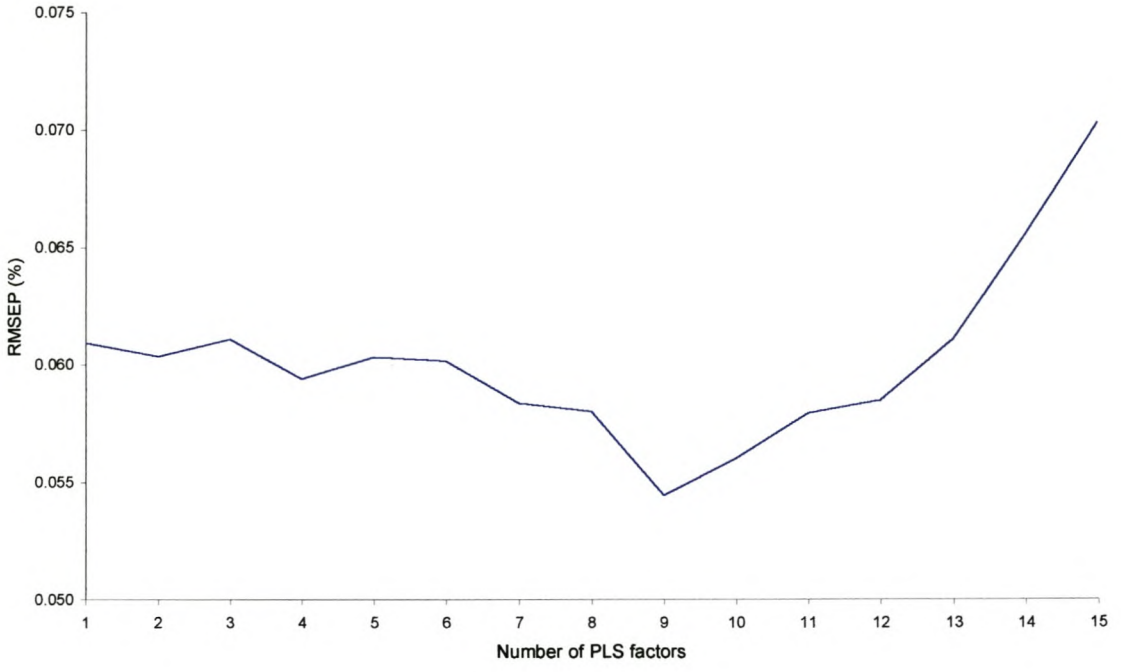

Figure 7.23. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the C18:3 calibration model.

Table 7.9. Statistical results for the C16:0, C18:0 and C18:1 calibration.

	C16:0 (raw data)	C18:0 (raw data)	C18:1 (*cis*) (2^{nd} D)
SEP	0.33%	1.63%	1.38%
Bias	0.02	-0.07	-0.11
RMSEP	0.33%	1.62%	1.38%
r	0.64	0.72	0.60
Elements (n):			
Calibration	200	200	200
Validation	98	98	98
Number of PLS factors	13	14	6
Mean	7.89%	35.62%	47.78%
Range	6.99 – 9.33%	30.98 – 40.81%	43.54 – 5.68%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

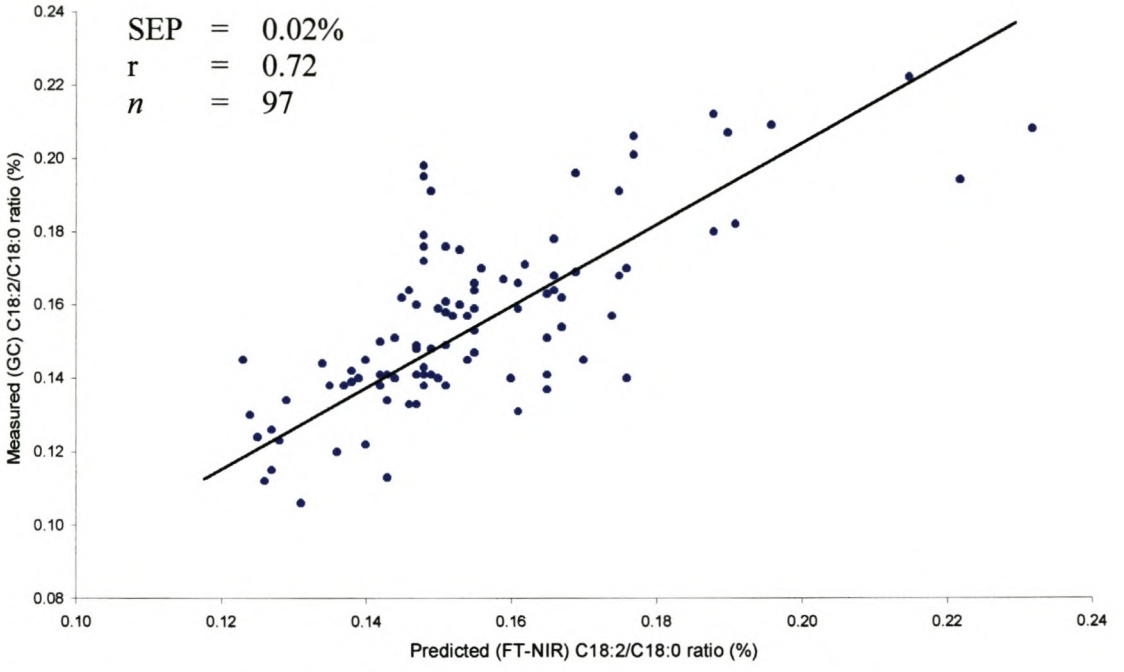

Figure 7.24. A validation plot of the predicted (FT-NIR) C18:2/C18:0 ratios versus the measured (reference value) C18:2/C18:0 ratios of mango kernel fat samples over a period of 240 days (raw data).

Table 7.10. Statistical results for the C18:2/C18:0 calibration (raw data).

SEP	0.02%
Bias	-0.001
RMSEP	0.02%
r	0.74
Elements (*n*):	
Calibration	201
Validation	97
Number of PLS factors	9
Mean	0.15%
Range	0.10 – 0.22%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

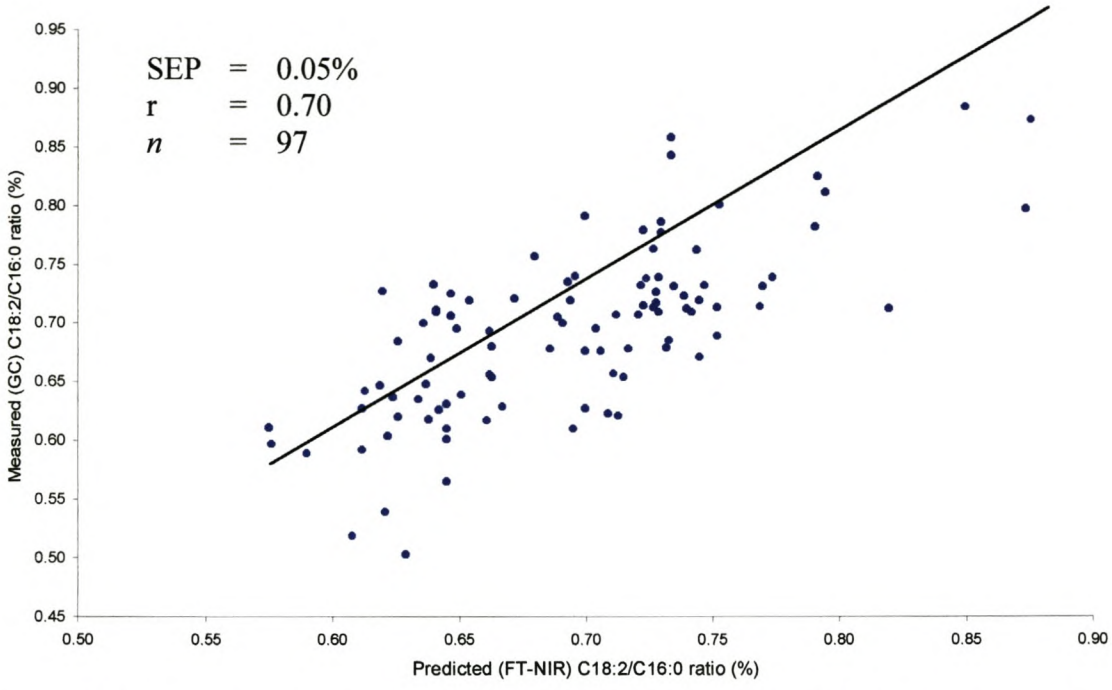

Figure 7.25. A validation plot of the predicted (FT-NIR) C18:2/C16:0 ratios versus the measured (reference value) C18:2/C16:0 ratios of mango kernel fat samples over a period of 240 days (raw data).

Table 7.11. Statistical results for the C18:2/C16:0 calibration (raw data).

SEP	0.05%
Bias	-0.001
RMSEP	0.05%
r	0.72
Elements (*n*):	
Calibration	201
Validation	97
Number of PLS factors	12
Mean	0.69%
Range	0.50 – 0.88%

SEP = Standard error of prediction
RMSEP = Root mean standard error of prediction
r = Correlation coefficient

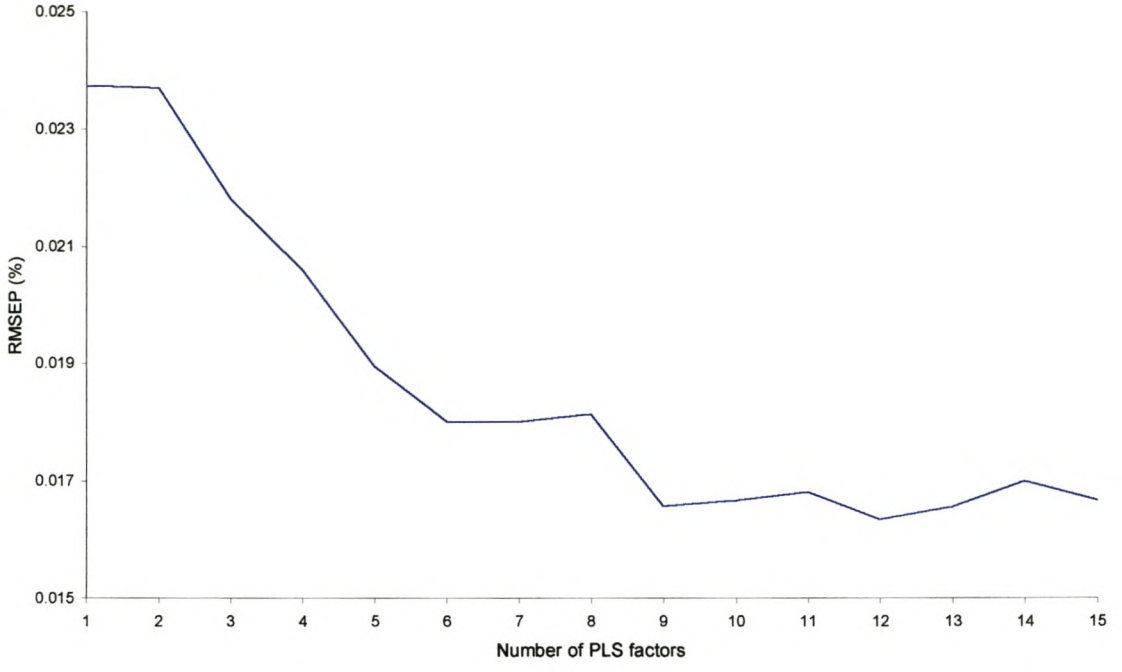

Figure 7.26. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the C18:2/C18:0 calibration model.

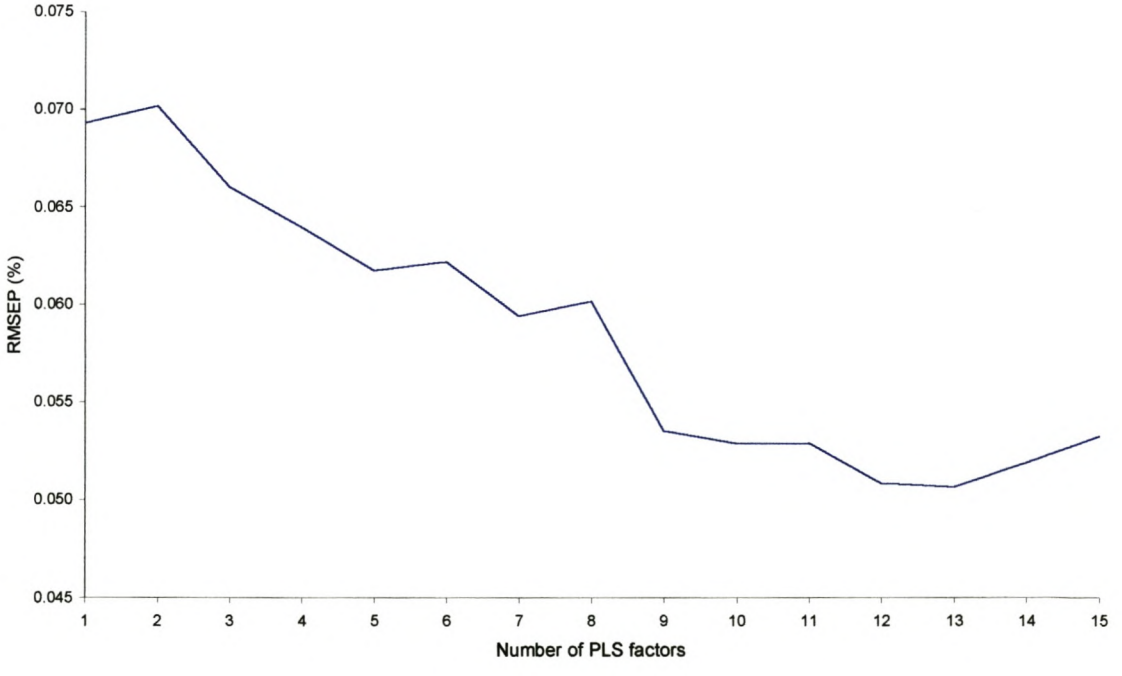

Figure 7.27. Residual validation variance plot for the standard error of prediction versus the number of partial least squares (PLS) factors used in the C18:2/C16:0 calibration model.

respectively. Good correlations were obtained for peroxide, conjugated diene and *p*-anisidine values and SEPs obtained were 0.46 meq.kg^{-1}, 0.01% and 0.32 mmol.kg^{-1}, respectively, indicating that the oxidative status of MKF can be successfully predicted by FT-NIRS when using PV, CD and AV as reference methods. When gas chromatography was implemented as a reference method, it was clear that good calibrations were only possible for the unsaturated fatty acids. Good correlations were found for C18:2 and C18:3 as well as for the ratios of C18:2/C18:0 and C18:2/C16:0. Weaker correlations were obtained for C18:1 (*cis*), C18:0 and C16:0 due to the stability of these fatty acids when compared to the spectral variation caused by the crude nature of the fat. Oxidative changes due to the alteration of fatty acid profile can successfully be predicted by Fourier transform near infrared spectroscopy. Furthermore, FT-NIR spectroscopy will be ideal for implementation as a screening method during the routine analysis of oxidative deterioration of MKF as part of a quality control system.

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CHAPTER 8

GENERAL DISCUSSION AND CONCLUSION

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The growth in world population as well as the scarcity of edible fats and oils in developing countries has led to the exploration of alternative sources of fats and oils (Hoffmann, 1989). This search for substitutes has motivated the nutritional and toxicological evaluation of products traditionally discarded as waste (Polasa & Rukmini, 1987) such as the kernel obtained from the stone of the mango fruit (Puravankara *et al.*, 2000). Although mango kernel fat (MKF) has already been evaluated for both nutritional and toxicological content and therefore declared safe for human consumption, successful application requires more research. The lack of information concerning the oxidative stability of mango kernel fat motivated this study and the aim was consequently to determine the oxidative stability of this fat when exposed to different storage conditions over a period of time.

Storage conditions included storage temperatures ranging from 5 to 40°C, emulsification of the MKF as well as exposure to oxygen and UV light. Application of the peroxide value (PV), conjugated diene value (CD) and *p*-anisidine value (AV) tests as well as gas chromatography (GC) and Fourier transform near infrared spectroscopy (FT-NIR) were applied to determine oxidative changes during storage at these conditions.

The peroxide value of the fresh MKF (2.7 $meq.kg^{-1}$) correlated relatively well with values obtained by Joseph (1995) (1.95 – 1.99 $meq.kg^{-1}$). During the 240-day trial, the PV of MKF never exceeded 5.17 $meq.kg^{-1}$ under any of the storage conditions and it was therefore assumed that no rancidity could be perceived. The conjugated diene values were also low compared to that of fats containing higher percentages of polyunsaturated fatty acids and the maximum CD value of 0.20% was determined for MKF when the samples were exposed to UV light. Measurement of secondary oxidation products with the AV test indicated a minimal production of 2-alkenals with values ranging between 0.5 and 5 $mmol.kg^{-1}$.

Gas chromatography analyses of the fatty acid methyl esters showed the predicted decline in unsaturated fatty acids. The stability of the saturated fatty acids was observed and determination of the C18:2/C18:0 as well as C18:2/C16:0 ratios, during the shelf life study, as recommended by Tan *et al.* (2001), showed the expected decrease. Although gas chromatography of fatty acid methyl esters could not

determine the oxidation products formed, the results obtained were very effectively combined with those of the PV, AV and CD value tests to produce a clear depiction of the oxidative status of the MKF. It can, however, be recommended that the implementation of headspace volatile analysis should be combined with these methods in future to determine the exact secondary products formed during oxidative deterioration.

Fourier transform near infrared calibration models were successfully developed to predict the oxidative status of mango kernel fat. Clear distinction was made between samples analysed on 0, 40 and the remaining (80 – 240) days when applying principal component analysis. The validation statistics for C18:2 showed a standard error of prediction (SEP) of 0.32% and a correlation coefficient of 0.82. Good correlations were also obtained for the C18:2/C16:0 as well as C18:2/C18:0 ratios. The calibration models for C18:1 (*cis*), C18:0 and C16:0 yielded weaker correlations due to the relative stability of these fatty acids to oxidation as shown by GC when compared to the spectral variation. It was also clear that the prediction of oxidative status was possible with NIR spectroscopy using the PV, CD and AV as reference methods. Inclusion of FT-NIR spectroscopy in a quality control system for monitoring the oxidative status of MKF has therefore been proven to be a useful alternative to analytical methods used for this purpose.

From the results obtained after the analysis of the oxidative stability of mango kernel fat, it may be proposed that it can be stored for a period of 8 months at temperatures up to 40°C, exposed to a limited amount of oxygen and UV light without developing rancidity. It would, however, be recommended that a shelf life study with varying concentrations of oxygen in the headspace as well as higher storage temperatures be included in future studies to establish the specific aldehydes and ketones formed during oxidation. The stabilising effect of emulsification of MKF was evident. According to these results, the MKF is stable against oxidative deterioration and therefore, this traditionally accepted waste product, can be recommended for use in products that require an extended shelf life.

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